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Abstract—The widespread and commercially important rougheye rockfish, *Sebastes aleutianus* (Jordan and Evermann, 1898), has been considered a single variable species, with light- and dark-colored forms, found on the outer continental shelf and upper slope of the North Pacific Ocean. Genetic analysis of 124 specimens verified the presence of two species in new specimens collected from Alaska to Oregon, and the two species were analyzed for distinguishing color patterns and morphological characters. Characters distinguishing the two were extended to an analysis of 215 additional formalin-fixed specimens representing their geographic ranges. *Sebastes aleutianus* is pale, often has dark mottling on the dorsum in diffuse bands, and does not have distinct dark spots on the spinous dorsal fin; it ranges from the eastern Aleutian Islands and southeastern Bering Sea to California. *Sebastes melanostictus* (Matsubara, 1934), the blackspotted rockfish, ranges from central Japan, through the Aleutian Islands and Bering Sea, to southern California. It is darker overall and spotting is nearly always present on the spinous dorsal fin. *Sebastes swifti* (Evermann and Goldsborough, 1907) is a synonym of *S. aleutianus*; *S. kawaradae* (Matsubara, 1934) is a synonym of *S. melanostictus*. The subgenus *Zalopyr* is restricted to *S. aleutianus* and *S. melanostictus*. Nomenclatural synonymies, diagnoses, descriptions, and distributions are provided for each species.

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Of the more than 110 species of *Sebastes* recognized worldwide, by far the greatest number of species are found in the North Pacific Ocean, where about 100 species are currently considered valid (Kai and Nakabo, 2002; Orr and Blackburn, 2004; Hyde and Vetter, 2007). Although very important commercially, members of the genus have a reputation for being difficult to identify, because they are similar in body shape and share other morphological characters. Live coloration is also an extremely important character used in diagnosis and identification. Molecular characters serve to identify most of these species and generally lend support to traditional morphological identifications and concepts of species limits. In addition, the use of genetic data has uncovered previously unrecognized species-level variation in what had been considered single species, as in the case of the rougheye rockfish complex, now known to comprise the two species *Sebastes aleutianus* (Jordan and Evermann, 1898) and *S. melanostictus* (Matsubara, 1934). The powerful combination of genetic and morphological analyses provides a means to identify the important characters useful in distinguishing new specimens, as well as archived material that, in some cases, has been preserved for over a century.

Ranging around the rim of the North Pacific Ocean and Bering Sea, from Japan to southern California, the species presently recognized as *Sebastes aleutianus* was originally described by Jordan and Evermann (1898) from four specimens taken in waters around Kodiak Island, Alaska. Evermann and Goldsborough (1907) later described *S. swifti*, a synonym of *Sebastes aleutianus*, from the northeastern Gulf of Alaska.

In the western Pacific, Matsubara (1934) described two species similar to *Sebastes aleutianus*, *S. melanostictus* and *S. kawaradae*. Barsukov (1964, 1970) determined that the names of both of these species were synonyms of *S. aleutianus*, apparently without examining type material. Kanayama and Kitagawa (1982) later examined the types and comparative material used by Matsubara (1934, 1943) as well as new material of *S. aleutianus* from the Bering Sea and Japanese Pacific coast. They also con-

cluded that both *S. melanostictus* and *S. kawaradae* are synonymous with *S. aleutianus*. These decisions were subsequently followed by Sheiko and Fedorov (2000), Love et al. (2002), Mecklenburg et al. (2002), and Nakabo (2002).

The confused history of the nomenclature of *Sebastes aleutianus* has been a result of the perceived wide variation in body coloration and close similarity in morphological features of several other species of *Sebastes*, including *S. borealis* (Barsukov, 1970), shortraker rockfish, and *S. melanostomus* (Eigenmann and Eigenmann, 1890), blackgill rockfish, in the eastern Pacific. Gilbert (1896) misidentified *S. aleutianus*, and probably *S. borealis*, material from the Bering Sea as that of *S. introniger* (Gilbert, 1890), which was originally described from southern California and is now considered a synonym of *S. melanostomus* (Phillips, 1957; Tsuyuki and Westrheim, 1970). In the same publication as their original description of *S. aleutianus*, Jordan and Evermann (1898) provided a species account of *S. introniger* that was primarily based on Gilbert's earlier (1896) redescription (Tsuyuki and Westrheim, 1970), again listing its range as including the Bering Sea. Until clarified by Barsukov (1970) and Tsuyuki and Westrheim (1970) with their original descriptions of the new species *S. borealis* Barsukov and its synonym *S. caenaematicus* Tsuyuki and Westrheim, these reports led to persistent records in the literature of *S. melanostomus* having been taken in the Bering Sea (Jordan and Evermann, 1898; Evermann and Goldsborough, 1907; Jordan et al., 1930; Barsukov, 1964; Allen and Smith, 1988), although it is a species that may range north only to extreme southeastern Alaska (Butler and Love, 2002; Mecklenburg et al., 2002; Love et al., 2002, 2005; Kramer and O'Connell, 2003).

The early work of Tsuyuki and coauthors (Tsuyuki et al., 1965, 1968; Tsuyuki and Westrheim, 1970) and Seeb (1986) on hemoglobin and allozymes of eastern Pacific *S. aleutianus* provided evidence for species-level differences among morphological and color forms—differences that were correlated with genetic variants. Using larger sample sizes and broader geographic ranges, Hawkins et al. (2005) with allozyme data and Gharrett et al. (2005) with DNA markers provided conclusive evidence of species-level differences among individuals in Alaskan waters, and the reality of the presence of two species within *S. aleutianus* was confirmed.

In this revision of the complex presently identified as *Sebastes aleutianus*, we recognize two species: *Sebastes aleutianus*, the rougheye rockfish, restricted to the eastern Pacific; and *Sebastes melanostictus*, the blackspotted rockfish, ranging across the North Pacific from Japan to California. *Sebastes kawaradae* is considered a synonym of *S. melanostictus*, and *S. swifti* is a synonym of *S. aleutianus*. We expand upon the efforts of Gharrett et al. (2006), who distinguished dark and light forms in the Gulf of Alaska, extending our morphological examination across almost the entire range of both species, clarifying the nomenclature, and providing diagnoses and redescriptions of both species.

Methods and materials

Counts, measurements, and statistical analyses followed Orr and Blackburn (2004), except as noted below. The structure of the swimbladder was examined after dissection following the method of Hallacher (1974). Unless indicated otherwise, standard length (SL) is used throughout, always measured from the tip of the snout. Measurements and counts are presented in species descriptions as the range for all material examined followed by the value for the holotype or lectotype in parentheses, when intraspecific variation is indicated.

Collection depths are noted for each cataloged lot in the *Material examined* section (see Appendix) when known. In the analysis of depth distributions, only those lots with a single depth reported were used; when a range of depths was reported for a lot, it was not included. All depths reported are bottom depths unless otherwise stated. Institutional abbreviations follow Eschmeyer (1998). Survey records for "rougheye rockfish" (*Sebastes aleutianus* or *S. melanostictus*) were taken from the Resource Assessment and Conservation Engineering Division, Alaska Fisheries Science Center (AFSC), database, which included catch data from groundfish surveys conducted from the Bering Sea and Aleutian Islands to southern California from 1961 to 2005.

During initial genetic analyses, 124 individuals were identified as *S. aleutianus* or *S. melanostictus* (=*S.* sp. cf. *aleutianus* of Hawkins et al., 2005) by using allozyme data following Hawkins et al. (2005) and are marked with an asterisk in the *Material examined* section (see Appendix). These specimens were collected primarily in the northern Gulf of Alaska and off Washington; two specimens, however, were collected from the eastern Aleutian Islands and northern California. Based on examination of these genetically identified individuals, significant differences were found in body color, specifically spotting on the spinous dorsal fin or an overall darker color (or both) in *S. melanostictus* (see *Species descriptions* below), that were then used to identify preserved specimens for which tissue was not available for genetic analysis. Allozyme characteristics and body color were used to group individuals for univariate tests, as well as for labeling individuals in graphs of principal components analysis scores. Nomenclature of allozyme protein variants and microsatellite alleles follows conventions of the American Fisheries Society (Shaklee et al., 1990). Statistical analyses were performed with Statgraphics Plus 4.1 (Manugistics, Rockville, MD), Splus 6.2 (Insightful Corp., Seattle, WA), and SPSS 11.5.1 (SPSS Inc., Chicago, IL). Differences were considered significant at $P < 0.05$.

Out of a total of 329 specimens examined morphologically, many had broken spines or other missing characters; thus, the data set was reduced to 137 specimens with complete data, including 40 of those genetically identified, for which both univariate and multivariate analyses could be conducted. A suite of 29 morphometric and 5 meristic characters was analyzed (Table 1). All

Table 1

Proportional morphometrics and meristics of *Sebastes aleutianus* (rougheye rockfish) and *Sebastes melanostictus* (blackspotted rockfish) for specimens in which all characters were used for multivariate analyses. Morphometric data are given in percent SL (standard length) and presented as the range, followed by the mean ± standard deviation (SD). *P* values are reported for statistically significant differences at α level of 0.05, as evaluated by ANCOVA or Kruskal-Wallis tests when appropriate; ns = not statistically significant at an α level of 0.05.

	Sebastes aleutianus	*Sebastes melanostictus*	
	Range (mean ±SD)	Range (mean ±SD)	*P*
n	57	80	
Standard length (mm)	77.1–470	101.6–445	
Meristics			
Dorsal-fin rays	12–14 (13.4 ±0.5)	13–15 (13.8 ±0.5)	<0.0001
Anal-fin rays	6–8 (7 ±0.3)	6–8 (7 ±0.2)	ns
Pectoral-fin rays (left)	17–19 (18.1 ±0.3)	17–19 (18.1 ±0.3)	ns
Lateral-line pores (left)	30–34 (31.2 ±0.9)	30–36 (32 ±1.2)	<0.0001
Gill rakers	29–33 (31.1 ±1.0)	30–35 (33 ±1.1)	<0.0001
Morphometrics			
Head length	34.6–42.5 (37 ±1.6)	34.6–41.4 (37.6 ±1.5)	
Orbit length	8.8–13.5 (10.7 ±1.1)	8.2–13.3 (10.4 ±1.0)	ns
Snout length	5.8–9.7 (7.5 ±0.7)	6.6–9.5 (7.9 ±0.6)	0.0486
Interorbital width	5.5–8.9 (7.1 ±0.7)	5.6–8.8 (7.4 ±0.7)	
Suborbital depth	1.6–2.9 (2.3 ±0.3)	1.5–3.3 (2.3 ±0.4)	0.0019
Upper jaw length	16.9–21.9 (19.2 ±0.9)	17.2–21.5 (19.2 ±0.7)	
Lower jaw length	20.4–27.2 (23.4 ±1.2)	20.7–26.8 (24.5 ±1.1)	
Gill raker length	3.4–6.1 (4.8 ±0.6)	3.9–6.8 (5.6 ±0.5)	<0.0001
Depth at pelvic-fin base	31.6–42.6 (36 ±1.9)	31.3–39.7 (34.9 ±1.8)	
Depth at anal-fin origin	23.9–31.9 (28 ±1.6)	23.5–29.4 (26.2 ±1.5)	<0.0001
Depth at anal-fin insertion	11.9–15.3 (13.9 ±0.7)	11.3–15.3 (13.3 ±1.0)	<0.0001
Dorsal-fin spine I length	4.6–7.2 (5.8 ±0.6)	6.1–9.6 (7.9 ±0.8)	<0.0001
Dorsal-fin spine IV length	10.6–15 (13.2 ±0.9)	12.3–18.9 (14.8 ±1.3)	<0.0001
Pectoral-fin base depth	8.4–10.4 (9.4 ±0.4)	8.2–10.4 (9.5 ±0.4)	ns
Pectoral-fin ray length	25–31 (27.4 ±1.3)	22–30 (26.3 ±1.4)	0.0013
Pelvic-fin ray length	19.3–25.1 (22.3 ±1.1)	19.4–25 (21.5 ±1.2)	0.0298
Pelvic-fin spine length	11.1–15.5 (13.5 ±1.2)	11.4–16.9 (13.8 ±1.2)	<0.0001
Anal-fin spine I length	4.7–9.4 (6.6 ±1.0)	4.7–10.1 (7.1 ±1.2)	<0.0001
Anal-fin spine II length	11–18.4 (14.4 ±1.8)	11.8–20.9 (15.2 ±2.2)	<0.0001
Anal-fin spine III length	14.2–19.8 (16.5 ±1.3)	12.4–20.9 (16.6 ±1.7)	<0.0001
Caudal peduncle depth	7.8–10.7 (9.7 ±0.5)	8.2–10.6 (9.2 ±0.5)	
Caudal peduncle dorsal length	10.5–15.3 (12.2 ±0.9)	11.3–15 (13 ±0.8)	
Caudal peduncle ventral length	17.8–21.9 (19.6 ±0.9)	17.4–21.7 (20.1 ±0.9)	
Preanal length	67.1–77.6 (71.8 ±2.5)	63.2–78.5 (70 ±2.6)	<0.0001
Predorsal length	31.2–39.5 (34.3 ±1.9)	31.6–38.4 (35 ±1.4)	
Spinous dorsal-fin-base length	31.9–41.7 (35.5 ±2.3)	32.4–40.9 (36.3 ±2.0)	ns
Soft dorsal-fin-base length	20.7–25.6 (22.9 ±1.2)	17.7–24.7 (21.5 ±1.4)	<0.0001
Anal-fin-base length	13.3–18.2 (15.3 ±0.9)	12.4–17 (14.2 ±1.0)	ns
Pre-pelvic-fin length	37.8–53.1 (44.4 ±4.2)	36.6–50.5 (42.4 ±2.5)	

morphometric characters were also tested for sexual dimorphism, where gender was treated as a categorical factor and the gender-species interaction was tested. Arcsine-transformed morphometric ratios (with SL or head length [HL] as denominator) were tested to meet the assumptions of normality and equality of variance required for ANCOVA. The following characters exhibited normal distributions and did not differ significantly in variance between species and thus were subjected to ANCOVA: orbit length, snout length, suborbital depth, gill-raker length, body depth at anal-fin origin, body depth at anal-fin insertion, dorsal-fin spine 1 length, dorsal-fin spine 4 length, pectoral-fin base depth, pectoral-fin ray length, pelvic-fin ray length, pelvic-fin

spine length, anal-fin spine 1 length, anal-fin spine 3 length, preanal length, spinous-dorsal-fin base length, soft-dorsal-fin base length, and anal-fin base length. Counts of meristic characters were tested by using the Kruskal-Wallis test.

To aid in distinguishing the two species, a standard principal component analysis (PCA) was conducted on both morphometric and meristic characters. The analyses were conducted on the covariance matrix of log-transformed raw morphometric data and the correlation matrix of raw meristic data. Differences between species were illustrated by plotting principal component (PC) 2 against PC3 of the morphometric analysis, PC1 against PC2 of the meristic analysis, and morphometric PC2 against meristic PC1.

Following the PCAs, a stepwise discriminant function analysis (DFA) was conducted by using morphometric and meristic data to establish the relative significance of those characters in distinguishing between the species. Morphometric data were standardized by dividing by standard length. Only characters meeting assumptions of multivariate normality and that exhibited statistically significant differences were analyzed. The robustness of the DFA was tested by conducting a leave-one-out cross-validation procedure (SPSS Inc., Chicago, IL).

For the data set containing all 329 specimens, only univariate analyses were conducted. The following characters, in addition to all those except orbit length in the reduced dataset, exhibited normal distributions, did not differ significantly in variance between species, and were subjected to ANCOVA: head length, lower-jaw length, body depth at pelvic-fin base, anal-fin spine 2 length, caudal-peduncle depth, caudal-peduncle dorsal length, caudal-peduncle ventral length, predorsal length, and pelvic-fin base to anal-fin origin length. Differences in counts of meristic characters were tested using the nonparametric Kruskal-Wallis test. With the exception of unbranched pectoral-fin rays, meristic data from specimens of all sizes were tested. In juveniles of less than 100 mm, all pectoral-fin rays were simple and counts for these specimens were not included in tests or presented in tables of lower pectoral-fin rays. Meristic data is presented in tables of frequency distributions by region. Four general regions were identified: 1) western Pacific Ocean, for material taken in Japanese and Russian waters; 2) Bering Sea and Aleutian Islands, for material from the eastern Bering Sea and Aleutian Islands to Unimak Pass; 3) Gulf of Alaska, from Unimak Pass to the Alaska-British Columbia border; and 4) the Lower West Coast, from Canada, Washington, Oregon, and California.

Results

Color pattern

Both in life and in preservation, body color differs consistently between *S. aleutianus* and *S. melanostictus* (Figs. 1–4 ; see detailed description below). The spinous dorsal fin in *S. melanostictus* is almost invariably discretely spotted. In most specimens, many small spots are scattered across the spinous dorsal fin, often continuing onto the soft dorsal fin; in some individuals, only two or three spots are present on the spinous dorsal fin. The presence or absence of these discrete spots was used in initial statistical analyses as the basis for identifying preserved specimens lacking tissue for genetic analysis. A few dark individuals without spots, or with spots apparently obscured by dark blotching, were aligned morphologically with other spotted individuals, and these dark individuals were also eventually identified as *S. melanostictus*. In contrast, all *S. aleutianus* examined were pale overall and lacked discrete spots, although often having diffuse mottling and blotches extending from the body onto the bases of the spinous and soft dorsal fins.

Morphometric and meristic characters

Specimens of *S. melanostictus* generally had longer dorsal-fin spines (Fig. 5, A and B), other spines, and gill rakers (Fig. 5C), whereas specimens of *S. aleutianus* had a deeper, more robust head and body and longer pelvic- and pectoral-fin rays. Among morphometric characters of specimens with all characters, snout length, suborbital depth, gill raker length, body depth at anal-fin origin, body depth at anal-fin insertion, dorsal-fin spine 1 length, dorsal-fin spine 4 length, pectoral-fin ray length, pelvic-fin ray length, pelvic-fin spine length, anal-fin spine 1 length, anal-fin spine 2 length, anal-fin spine 3 length, preanal length, and soft-dorsal-fin base length differed significantly between *S. aleutianus* and *S. melanostictus* (Table 1). In addition, among specimens of the larger data set, head length, lower jaw length, depth at pelvic-fin base, caudal-peduncle depth, caudal-peduncle dorsal length, and caudal-peduncle ventral length also differed significantly, and anal-fin spine 1 and 3 lengths became nonsignificant (Table 2).

Plots of PCA scores revealed prominent differences between the two species among morphometric characters, as well as slight differences among meristic characters. In the morphometric PCA, all characters were positively loaded on PC1 (the size component; Table 3), which explained 96.8% of the total variation. Among the principal shape components, PC2 explained 1.3% of variation and was heavily loaded on dorsal-fin spine 1 length, gill-raker length, anal-fin spine 1 length, anal-fin spine 2 length, and dorsal-fin spine 4 length (Table 3); PC3 explained 0.3% of variation and was heavily loaded on anal-fin spine 1 length, suborbital depth, gill-raker length, prepelvic length, and orbit length (Table 3). In the plot of PC2 versus PC3, the two clusters representing *S. aleutianus* and *S. melanostictus* overlapped narrowly; 16 (8 *S. aleutianus*, 8 *S. melanostictus*) of 137 individuals examined were in this area of overlap (Fig. 6A). Of 40 genetically identified individuals, three *S. aleutianus* and three *S. melanostictus* were included in the overlap area. One

Figure 1

Sebastes aleutianus (rougheye rockfish), UW 116468, 120.2–138.6 mm, females, northern Gulf of Alaska, 59.6918°N, 148.8569°W, 180 m depth, 28 July 2005. Photo by J. W. Orr.

specimen with an unknown genotype, a possible hybrid, was also included.

Although meristic characters broadly overlapped in ranges, several characters were indicative of a species-level difference between *S. aleutianus* and *S. melanostictus*. Numbers of gill rakers, lateral-line pores, and dorsal-fin rays differed significantly (Tables 1 and 2). No regional or clinal differences were evident in frequency data (Tables 4 and 5). Significant differences were also expressed in the meristic PCA as heavy loadings on PC1 (Table 6), which, however, revealed only a slight separation between broadly overlapping clusters of the two species when scores were plotted for PC1 and PC2 (Fig. 6B).

Plotting the scores of PC1 of the meristic analysis versus PC2 of the morphometric analysis resulted in slightly better resolution between the two species clusters (Fig. 6C). In contrast with the morphometrics-only plot that had 16 individuals in the area of overlap and the meristics-only plot in which the clusters nearly completely overlapped, only nine individuals were found in the area of overlap when the morphometric PC2 was plotted against the meristic PC1. As in the morphometrics-only plot, six of these individuals were genetically identified.

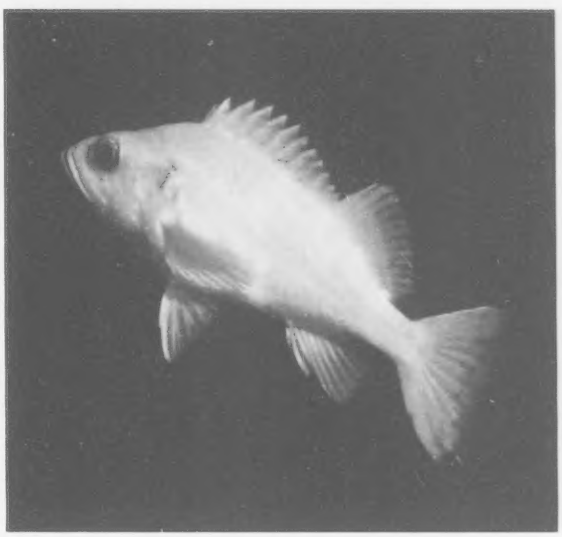

Figure 2

Underwater photo of *Sebastes aleutianus* (rougheye rockfish) taken south of Kodiak Island, Alaska, ca. 56°N, 153°N, July 2005. Photo by D. Hanselman.

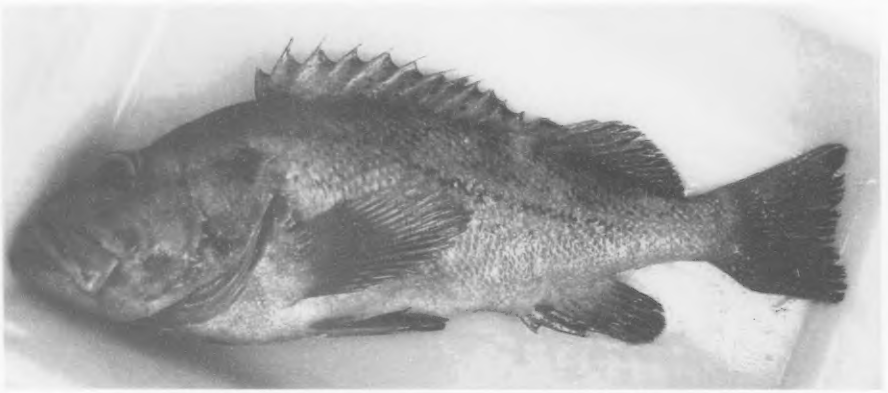

Figure 3

Sebastes melanostictus (blackspotted rockfish), UW 48464, 249.5 mm, male, Aleutian Islands, north of Islands of Four Mountains, 53.2207°N, 169.7395°W, 328 m depth, 2 June 2002. Photo by J. W. Orr.

Discriminant function analysis

Although the PCA produced overlapping species clusters of individuals identified and labeled separately from the analysis, the discriminant function analysis verified the *a priori* identification of nearly all individuals. The single linear discriminant function equation produced was highly significant (Wilks's λ=0.197, χ^2=222.772, 8 df, P<0.0001):

$$D = 101.557(d1) + 52.453(snl) + 0.294(gr) + 51.92(grl) + 0.564(dr) - 38.604(p2rl) - 22.601(d2b) - 10.203(pal) - 10.445,$$

where D = the discriminant score of an individual;
$d1$ = length of dorsal-fin spine 1 divided by SL;
snl = snout length divided by SL;
gr = number of gill rakers;
grl = length of gill rakers divided by SL;
dr = number of dorsal-fin rays;
$p2rl$ = length of pelvic-fin rays divided by SL;
$d2b$ = length of soft-dorsal-fin base; and
pal = preanal length divided by SL.

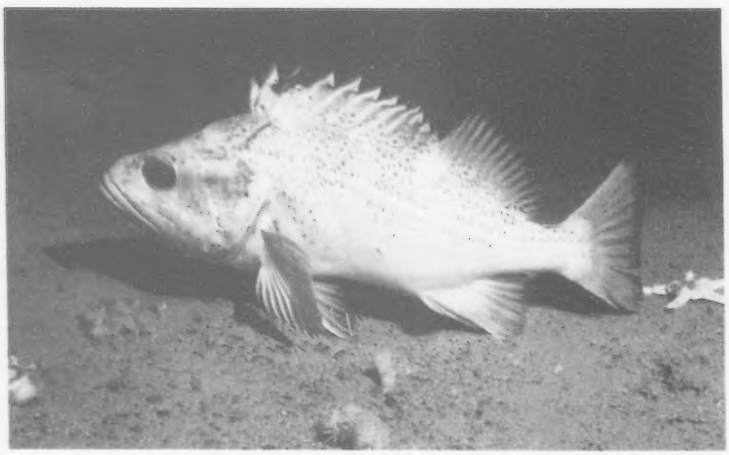

Figure 4

Underwater photo of *Sebastes melanostictus* (blackspotted rockfish) taken south of Kodiak Island, Alaska, ca. 56°N, 153°N, July 2005. Photo by D. Hanselman.

All but two individuals with negative scores had been previously identified as *S. aleutianus*, whereas all but one individual with positive scores were *S. melanostictus*. The discriminant function equation, therefore, correctly classified 97.8% of individuals. The possible hybrid was classified correctly as *S. aleutianus*. The cross-validation procedure correctly classified 97.1% of individuals. In addition to the three individuals above, one *S. aleutianus* was also misclassified as *S. melanostictus* in cross validation. When using this equation for future species

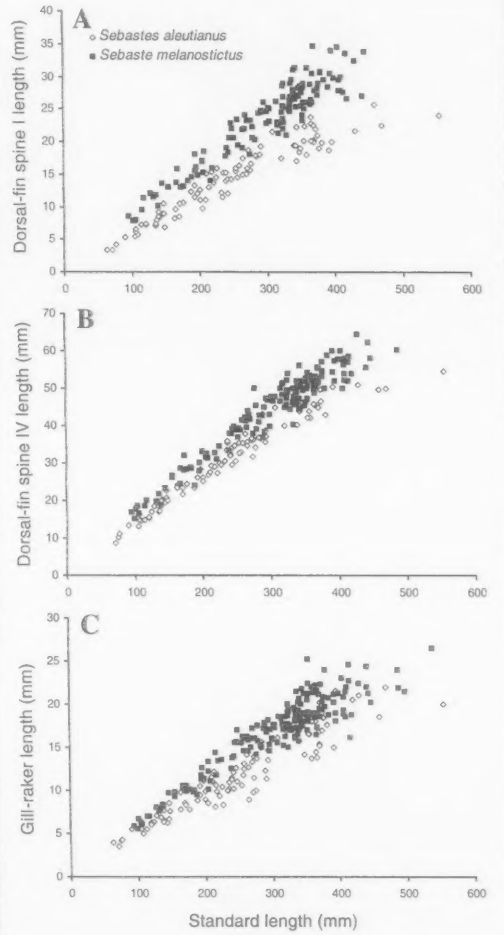

Figure 5

Plots of diagnostic morphometric characters versus standard length of *Sebastes aleutianus* (rougheye rockfish, open diamond) and *S. melanostictus* (blackspotted rockfish, closed square). (**A**) Dorsal-fin spine I length, (**B**) dorsal-fin spine IV length, and (**C**) gill raker length. The first and fourth dorsal-fin spines and gill rakers are longer in most specimens of *S. melanostictus* than in *S. aleutianus*.

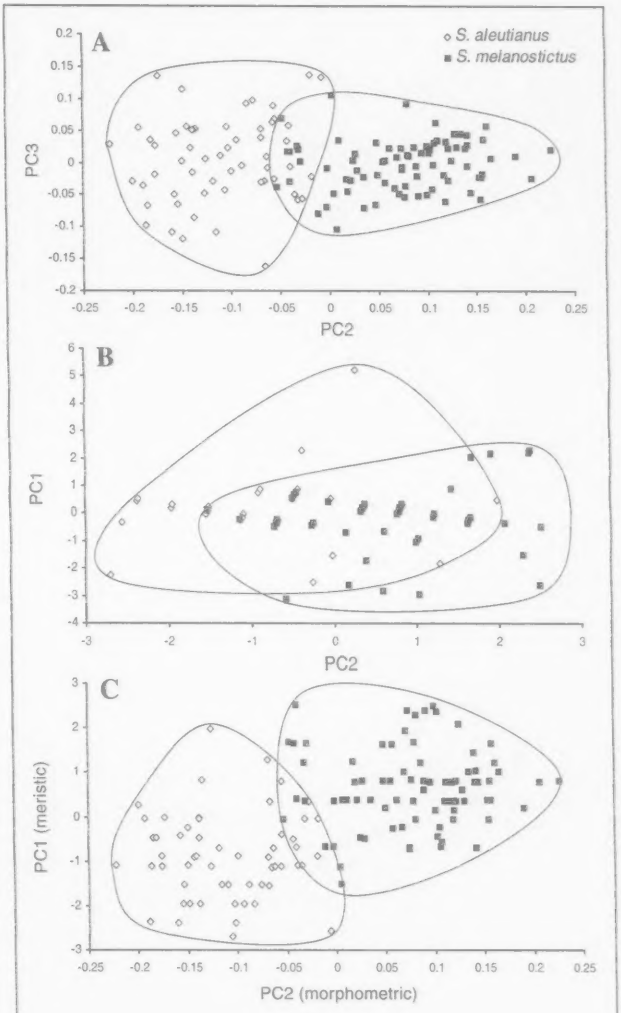

Figure 6

Plots of principal component (PC) scores for morphometric and meristic characters for *Sebastes aleutianus* (rougheye rockfish, open diamond) and *S. melanostictus* (blackspotted rockfish, closed square). (**A**) Morphometric characters only, (**B**) meristic characters only, and (**C**) morphometric (PC2) versus meristic characters (PC1). Differences among specimens along the major axis of dispersion in the morphometric analysis (PC2) were primarily due to longer dorsal-fin spines, anal-fin spines, and gill rakers in *S. melanostictus*. The slight separation among specimens along the major axis in the meristic analysis (PC2) was primarily due to higher counts of gill rakers, lateral-line pores, and dorsal-fin rays in *S. melanostictus*.

separation, one would identify individuals with a score above 0 as *S. melanostictus* and those below 0 as *S. aleutianus*.

Systematics

Sebastes aleutianus (Jordan and Evermann, 1898) Rougheye rockfish

Figures 1, 2, 5–8; Tables 1, 2, 4, 5

Sebastodes aleutianus Jordan and Evermann, 1898:1795, pl. 16, figs. 1–4 (original description, four specimens: lectotype herein designated USNM 48800, male, 374.2 mm; paralectotypes, three specimens 370–470 mm, SU 12928, *Albatross* station 3676, Shelikof Strait, Alaska, off Karluk, Kodiak Island, 223 m depth, 20 July 1897).

Table 2

Proportional morphometrics and meristics of *Sebastes aleutianus* (rougheye rockfish) and *Sebastes melanostictus* (blackspotted rockfish) for all specimens examined. Morphometric data are given in percent SL (standard length) and presented as the range, followed by the mean ± standard deviation (SD). *P* values are reported for statistically significant differences at 0.05 level, as evaluated by ANCOVA or Kruskal-Wallis tests when appropriate; ns = not statistically significant at an α level of 0.05.

	Sebastes aleutianus		*Sebastes melanostictus*		
	n	Range (mean ±SD)	*n*	Range (mean ±SD)	*P*
Standard length (mm)	117	63.4–555.2	193	95.5–539	
Meristics					
Dorsal-fin spines	114	12–14 (13.0 ±0.2)	182	12–14 (13.0 ±0.2)	ns
Dorsal-fin rays	109	12–14 (13.5 ±0.5)	184	13–15 (13.7 ±0.5)	<0.001
Anal-fin rays	108	6–8 (7.1 ±0.3)	182	6–8 (7.1 ±0.3)	ns
Pectoral-fin rays	106	17–19 (18.1 ±0.3)	170	17–19 (18.1 ±0.4)	ns
Unbranched pectoral-fin rays	99	4–9 (7.7 ±0.9)	177	5–10 (7.9 ±0.7)	ns
Lateral-line pores	105	30–34 (31.4 ±1.0)	184	30–36 (32.0 ±1.2)	<0.001
Gill rakers	116	29–34 (31.2 ±1.0)	191	30–36 (33.0 ±1.2)	<0.001
Gill rakers of upper arch	98	8–10 (9.1 ±0.5)	165	8–11 (9.6 ±0.6)	<0.001
Gill rakers of lower arch	98	21–24 (22.1 ±0.8)	165	21–26 (23.4 ±0.8)	<0.001
Morphometrics					
Head length	106	33.5–41.4 (36.8 ±1.4)	179	34–41.4 (37.6 ±1.4)	0.006
Orbit length	93	8.7–13.5 (10.7 ±1.1)	145	8.2–13.3 (10.2 ±0.9)	
Snout length	93	5.7–9.7 (7.5 ±0.7)	143	6.2–9.6 (8 ±0.6)	<0.001
Interorbital width	93	5.5–8.9 (7.2 ±0.7)	141	5.6–9.2 (7.5 ±0.7)	
Suborbital depth	90	1.6–2.9 (2.3 ±0.3)	141	1.5–3.3 (2.3 ±0.3)	ns
Upper jaw length	89	16.9–21.1 (19.1 ±0.7)	141	17.2–21.5 (19.2 ±0.8)	
Lower jaw length	91	20.4–27.2 (23.6 ±1.2)	138	21.6–27.1 (24.6 ±1.0)	<0.001
Gill raker length	106	3.4–6.3 (4.9 ±0.6)	178	3.9–7.1 (5.6 ±0.6)	<0.001
Depth at pelvic-fin base	89	31.6–42.6 (36.1 ±1.9)	143	31.3–40.1 (35.3 ±1.9)	0.005
Depth at anal-fin origin	97	23.5–31.9 (27.9 ±1.6)	154	22.8–30.5 (26.6 ±1.6)	<0.001
Depth at anal-fin insertion	90	11.4–15.6 (13.8 ±0.8)	144	11.3–15.6 (13.5 ±0.9)	0.003
Dorsal-fin spine I length	91	4.3–7.2 (5.8 ±0.6)	135	5.9–9.6 (7.8 ±0.7)	<0.001
Dorsal-fin spine IV length	97	9.8–15.5 (13.2 ±1.0)	151	12–18.4 (14.8 ±1.2)	<0.001
Dorsal-fin ray length	82	13.5–20 (16.6 ±1.3)	135	10.4–18.8 (15.5 ±1.3)	
Pectoral-fin base depth	88	8.4–10.4 (9.4 ±0.4)	142	8.2–10.4 (9.5 ±0.4)	ns
Pectoral-fin ray length	90	24.4–31 (27.3 ±1.3)	140	22–30 (26.3 ±1.4)	<0.001
Pelvic-fin ray length	92	19.3–25.1 (22.1 ±1.1)	159	18.8–25 (21.4 ±1.2)	<0.001
Pelvic-fin spine length	103	9.5–16.6 (13.4 ±1.3)	160	9.6–16.9 (13.7 ±1.2)	ns
Anal-fin spine I length	94	4.5–9.7 (6.8 ±1.1)	156	4.5–10.1 (7 ±1.1)	ns
Anal-fin spine II length	94	11–18.4 (14.5 ±1.9)	147	11.8–19.6 (14.9 ±1.9)	ns
Anal-fin spine III length	95	13–20.2 (16.6 ±1.5)	153	12.2–21.3 (16.5 ±1.7)	ns
Anal-fin ray 1 length	63	16–24.2 (19.5 ±1.6)	119	15.3–25 (18.9 ±1.8)	
Anal-fin ray 2 length	62	17.4–26.4 (21.1 ±1.8)	115	16.7–26.9 (19.9 ±2.0)	
Last anal-fin ray length	62	6–12.6 (10.4 ±1.1)	113	8.3–13.7 (10.4 ±0.8)	
Caudal peduncle depth	90	7.8–10.7 (9.6 ±0.5)	141	7.9–10.6 (9.2 ±0.5)	<0.001
Caudal peduncle dorsal length	87	10.5–13.9 (12 ±0.8)	141	11.1–15 (12.9 ±0.7)	<0.001
Caudal peduncle ventral length	89	17.7–21.9 (19.5 ±0.9)	141	17.4–22.5 (20.1 ±1.0)	<0.001
Preanal length	89	67.1–77.6 (71.8 ±2.3)	141	63.2–78.5 (70.2 ±2.6)	<0.001
Predorsal length	90	30.3–39.3 (34.3 ±1.7)	141	31.6–38.4 (34.9 ±1.4)	ns
Pelvic-fin base to anal-fin origin length	87	25.9–37 (30.8 ±2.6)	139	23.8–38.6 (30.9 ±2.9)	ns
Spinous dorsal-fin-base length	91	30.8–41.7 (35.7 ±2.3)	141	32.4–41.2 (36.4 ±2.0)	ns
Soft dorsal-fin-base length	90	20.2–25.8 (22.8 ±1.2)	141	17.3–24.7 (21.4 ±1.5)	<0.001
Anal-fin base length	89	13.1–18.2 (15.2 ±0.9)	141	12.4–17 (14.2 ±1.0)	
Pre-pelvic-fin length	87	37.8–53.1 (44.2 ±3.8)	139	36.6–52.6 (42.6 ±2.8)	
Caudal-fin length	80	20–28.1 (24 ±1.5)	128	19.2–25.2 (22 ±1.2)	

Sebastodes swifti Evermann and Goldsborough, 1907:285, fig. 36 (original description, two specimens: holotype, USNM 57821, sex unknown, 128.2 mm, *Albatross* station 4234, Yes Bay, Alaska; paratype, USNM 126656, sex unknown, 75.2 mm, *Albatross* station 4246, Kasaan Bay, Alaska, 185–225 m depth, 11 July 1903).

Zalopyr aleutianus: Jordan et al., 1930:365 (new combination).

Sebastes aleutianus: Barsukov, 1970:997 (new combination; comparison with *S. borealis*).

Diagnosis

This species of *Sebastes* is distinguished from all other species except *S. melanostictus* in having eight pairs of head spines and two or more infraorbital spines. It is distinguished from *S. melanostictus* by the following combination of color patterns and morphological and genetic characteristics: body pale, distinct spots absent on the membranes of the spinous dorsal fin; dorsal-fin spine 1 shorter, 1.5–3.0 times into orbit length, 4.3–7.2% SL (vs. 1.0–1.8 times into orbit length and 5.9–9.6% SL); dorsal-fin spine 4 shorter, 9.8–15.5% SL (vs. 12.0–18.4% SL); gill rakers shorter, 3.4–6.3% SL (vs. 3.9–7.1% SL), and fewer, 29–34 (vs. 30–36) (Table 2). Genetically diagnosed by a combination of the presence of protein variants *ACP*100*, either *IDDH*100* or **500* or both, either *PGM-2*100* or **83* or both, and *XO*100* (Hawkins et al., 2005); homozygosity at microsatellite allele *μSma6*183* (Gharrett et al., 2005, 2006); and mitochondrial haplotype B of Gharrett et al. (2005, 2006).

Description

D XII–XIV (XIII), 12–14 (14); A III, 6–8 (8); P1 17–19 (18), 4–9 (6) simple; lateral-line pores 30–34 (31), scales 42–57; infraorbital spines 2–10 (9); gill rakers 29–34 (8–10 + 21–24) (31:9+22); vertebrae 27 (10+17); pyloric caeca 9–12. Meristic frequency and statistical data are presented in Tables 1, 2, 4, and 5.

Table 3

Factor loadings for principal component (PC) analysis of morphometric characters of *Sebastes aleutianus* (rougheye rockfish) and *Sebastes melanostictus* (blackspotted rockfish), in which all characters were used for multivariate analyses.

	PC1	PC2	PC3
Head length	0.1911	−0.0190	−0.1584
Orbit length	0.1538	0.0215	−0.2248
Snout length	0.2020	−0.0121	−0.2133
Interorbital width	0.2261	−0.0922	−0.0498
Suborbital depth	0.2288	−0.2145	0.3787
Upper jaw length	0.1936	−0.0528	−0.0943
Lower jaw length	0.2026	0.0024	−0.1023
Gill raker length	0.1753	0.3399	−0.3728
Depth at pelvic-fin base	0.1960	−0.1374	−0.0036
Depth at anal-fin origin	0.1944	−0.2055	0.0864
Depth at anal-fin insertion	0.1929	−0.1621	0.0306
Dorsal-fin spine I length	0.1982	0.6188	0.1775
Dorsal-fin spine IV length	0.1755	0.2379	−0.0909
Pectoral-fin base width	0.1905	−0.0489	0.0642
Pectoral-fin ray length	0.1741	−0.0983	−0.0211
Pelvic-fin ray length	0.1746	−0.0690	−0.0030
Pelvic-fin spine length	0.1654	0.0766	0.0457
Anal-fin spine I length	0.1417	0.3270	0.4728
Anal-fin spine II length	0.1369	0.2472	0.1530
Anal-fin spine III length	0.1586	0.1029	0.1761
Caudal peduncle depth	0.1913	−0.1736	0.0632
Caudal peduncle dorsal length	0.1975	0.0578	−0.1842
Caudal peduncle ventral length	0.1869	0.0142	−0.1128
Preanal length	0.1881	−0.0926	0.0627
Predorsal length	0.1876	−0.0057	−0.2195
Spinous dorsal-fin-base length	0.1957	−0.0223	−0.2150
Soft dorsal-fin-base length	0.1804	−0.1481	0.0548
Anal-fin-base length	0.1654	−0.1322	0.1176
Pre-pelvic-fin length	0.1874	−0.1256	0.2757

Table 4

Counts of dorsal-fin spines, soft-dorsal-fin rays, anal-fin rays, and total and unbranched pectoral-fin rays in *Sebastes aleutianus* (rougheye rockfish) and *Sebastes melanostictus* (blackspotted rockfish). Lower West Coast = waters off the coast of British Columbia, Washington, Oregon, and California. Western Pacific Ocean = waters off the coast of the Kuril Islands and Japan.

	Dorsal-fin spines			
	12	13	14	*n*
Sebastes aleutianus				
Lower West Coast		29	1	
Gulf of Alaska	1	63	2	
Bering Sea and Aleutian Is.		18		
Total	1	110	3	114
Sebastes melanostictus				
Lower West Coast		17	1	
Gulf of Alaska	1	80	4	
Bering Sea and Aleutian Is.	2	53		
Western Pacific Ocean		24		
Total	3	174	5	182

	Dorsal-fin rays				
	12	13	14	15	*n*
Sebastes aleutianus					
Lower West Coast		11	19		
Gulf of Alaska	1	36	30		
Bering Sea and Aleutian Is.		5	13		
Total	1	52	62		115
Sebastes melanostictus					
Lower West Coast		5	13		
Gulf of Alaska		32	53		
Bering Sea and Aleutian Is.		17	37	1	
Western Pacific Ocean		6	14	4	
Total		60	117	5	182

	Anal-fin rays			
	6	7	8	*n*
Sebastes aleutianus				
Lower West Coast	1	29		
Gulf of Alaska	1	59	6	
Bering Sea and Aleutian Is.		10	2	
Total	2	98	8	108
Sebastes melanostictus				
Lower West Coast		17	1	
Gulf of Alaska	2	75	7	
Bering Sea and Aleutian Is.		53	5	
Western Pacific Ocean		20		
Total	2	165	13	180

	Total left pectoral-fin rays				
	17	18	19	*n*	
Sebastes aleutianus					
Lower West Coast		30			
Gulf of Alaska	2	55	7		
Bering Sea and Aleutian Is.		9	3		
Total	2	94	10	106	
Sebastes melanostictus					
Lower West Coast		16	1		
Gulf of Alaska	2	72	12		
Bering Sea and Aleutian Is.	4	50	4		
Western Pacific Ocean		20			
Total	6	158	17	181	

	Unbranched left pectoral-fin rays							
	4	5	6	7	8	9	10	*n*
Sebastes aleutianus								
Lower West Coast			1	3	20	4		
Gulf of Alaska	1	1	5	9	39	4		
Bering Sea and Aleutian Is.			1	5	4	2		
Total	1	1	7	17	63	10		99
Sebastes melanostictus								
Lower West Coast		1		5	9		1	
Gulf of Alaska				10	58	13		
Bering Sea and Aleutian Is.		1		9	38	10		
Western Pacific Ocean					8	10	2	
Total		2		32	115	25	1	175

Body robust, depth at pelvic-fin base 31.6–42.6 (38.1) % SL; relatively deep at anal-fin origin 23.5–31.9 (28.0) % SL; profile of dorsal margin of head gently sloping from dorsal-fin origin to snout, dorsal rim of orbit included in lateral margin of frontals. Mouth large, with posterior end of maxilla extending between pupil and posterior rim of orbit to just beyond the posterior rim of the orbit; maxilla length 16.9–21.1 (19.9) % SL; symphyseal knob moderately pronounced with blunt tip, lower-jaw length 20.4–27.2 (25.0) % SL; mandibular pores large to moderate in size. Cranial spines strong, often rough. Nasal, preocular, supraocular, postocular, tympanic, coronal, parietal, and supratemporal spines invariably present; all major head spines strong, except for coronal spines, which are weak; supraocular and postocular spines and parietal ridge often rough;

Table 5

Counts of gill rakers, lateral-line pores, and left infraorbital spines in *Sebastes aleutianus* (rougheye rockfish) and *Sebastes melanostictus* (blackspotted rockfish). Lower West Coast = waters off the coast of British Columbia, Washington, Oregon, and California. Western Pacific Ocean = waters off the coast of the Kuril Islands and Japan.

	Gill rakers								
	29	30	31	32	33	34	35	36	*n*
Sebastes aleutianus									
Lower West Coast	1	8	15	6	3				
Gulf of Alaska	5	8	28	19	6	2			
Bering Sea and Aleutian Is.		5	4	6					
Total	6	16	43	25	9	2			101
Sebastes melanostictus									
Lower West Coast		1	3	3	4	7	1		
Gulf of Alaska		1	9	25	22	26	10		
Bering Sea and Aleutian Is.		1	7	12	24	10	3	2	
Western Pacific Ocean		1		5	6	5	3		
Total		4	19	45	56	48	17	2	191

	Gill rakers of the upper arch				
	8	9	10	11	*n*
Sebastes aleutianus					
Lower West Coast	4	21	4		
Gulf of Alaska	3	39	13		
Bering Sea and Aleutian Is.	4	10			
Total	11	70	17		98
Sebastes melanostictus					
Lower West Coast		2	12	1	
Gulf of Alaska	2	32	40	6	
Bering Sea and Aleutian Is.	3	21	23	1	
Western Pacific Ocean		9	9	2	
Total	5	64	84	10	163

	Gill rakers of the lower arch						
	21	22	23	24	25	26	*n*
Sebastes aleutianus							
Lower West Coast	7	14	7	1			
Gulf of Alaska	10	31	10	4			
Bering Sea and Aleutian Is.	2	6	6				
Total	19	51	23	5			98
Sebastes melanostictus							
Lower West Coast		2	5	8			
Gulf of Alaska	1	9	25	40	5		
Bering Sea and Aleutian Is.	1	6	18	18	4	1	
Western Pacific Ocean	1	3	8	7	1		
Total	3	20	56	73	10	1	163

continued

sphenotic spines often prominent, occasionally moderate or small in size. Interorbital region wide, 5.5–8.9 (8.7) % SL, flat to slightly concave; frontal ridges between orbits moderate to strong; parietal ridges strong, with area between ridges slightly convex or flat. Preopercular spines 5, directed posteroventrally; opercular spines 2, upper spine directed posteriorly, lower spine directed posteriorly and slightly ventrally. Posttempo-

Table 5 (continued)

	Lateral-line pores							
	30	31	32	33	34	35	36	*n*
Sebastes aleutianus								
Lower West Coast	2	17	9	1	1			
Gulf of Alaska	8	32	15	5	2			
Bering Sea and Aleutian Is.	2	3	4	2	2			
Total	12	52	28	8	5			105
Sebastes melanostictus								
Lower West Coast	1	6	1	6	2			
Gulf of Alaska	7	29	37	13	2	1		
Bering Sea and Aleutian Is.	3	12	24	12	5		1	
Western Pacific Ocean		5	6	3	3	1	2	
Total	11	52	68	34	12	2	3	182

	Left infraorbital spines											
	2	3	4	5	6	7	8	9	10	11	12	*n*
Sebastes aleutianus												
Lower West Coast	1	9	8	6	4	3	1					
Gulf of Alaska	2	10	13	10	6	8	3	2	2			
Bering Sea and Aleutian Is.		1	2	6	3	1						
Total	3	20	23	22	13	12	4	2	2			101
Sebastes melanostictus												
Lower West Coast		2	1	5	5		1	1			1	
Gulf of Alaska	4	9	9	12	17	5	11	2	3	3		
Bering Sea and Aleutian Is.	5	11	4	11	6	5	2		1	1		
Western Pacific Ocean			1	3	4	1	1	1				
Total	9	22	15	31	32	11	15	4	4	4	1	148

	Left infraorbital 1 spines					
	0	1	2	3	4	*n*
Sebastes aleutianus						
Lower West Coast	1	22	8			
Gulf of Alaska	5	28	15	7		
Bering Sea and Aleutian Is.	3	8	2			
Total	9	58	25	7		99
Sebastes melanostictus						
Lower West Coast	1	2	9	4		
Gulf of Alaska	2	29	29	9	6	
Bering Sea and Aleutian Is.	2	24	15	4		
Western Pacific Ocean		1	9	1		
Total	5	56	62	18	6	147

continued

ral and supracleithral spines present. Ventral margin of lachrymal typically with two rounded lobes, both anterior and posterior often with a single small posteriorly directed spine, posterior occasionally with as many as 4 or 5 small spines forming a serrate margin; infraorbital spines small, 2–10 (9): 0–3 (3) on first infraorbital, 0–5 (5) on second, and 1–3 (1) on third; dorsal margin of opercle nearly horizontal; lower margin of gill cover with 1 or 2 small spines, produced by posteroventral tip of interopercle and anteroventral tip of subopercle.

Dorsal-fin origin above anterodorsal portion of gill slit; dorsal fin continuous, gradually increasing in height from spine I (4.3–7.2% SL, 1.5–3.0 times into

Table 5 (continued)

	Left infraorbital 2 spines								
	0	1	2	3	4	5	6	*n*	
Sebastes aleutianus									
Lower West Coast		11	15	4		1			
Gulf of Alaska	1	14	25	10	2	3			
Bering Sea and Aleutian Is.		1	9	2	1				
Total	1	26	49	16	3	4		99	
Sebastes melanostictus									
Lower West Coast	1	5	6	2	1		1		
Gulf of Alaska	4	14	31	13	9	3	1		
Bering Sea and Aleutian Is.	3	16	20	4	1		1		
Western Pacific Ocean		1	7	2	1				
Total	8	36	64	21	12	3	3	147	

	Left infraorbital 3 spines								
	0	1	2	3	4	5	6	7	*n*
Sebastes aleutianus									
Lower West Coast		18	12	1					
Gulf of Alaska		26	25	4					
Bering Sea and Aleutian Is.		2	10	1					
Total		46	47	6					99
Sebastes melanostictus									
Lower West Coast		7	8	1					
Gulf of Alaska	3	27	38	6	1				
Bering Sea and Aleutian Is.	1	18	22	2	1			1	
Western Pacific Ocean		3	7	1					
Total	4	55	75	10	2	0	0	1	147

orbit length; Fig. 5A) to spine IV (9.8–15.5% SL; Fig. 5B; tips of dorsal-fin spines broken in lectotype) and decreasing in height to spine XII; spine XIII much longer, forming anterior support of dorsal-fin rays; membranes of spinous dorsal fin moderately incised, less so posteriorly; soft dorsal fin with anterior rays longest, posterior rays gradually shortening. Anal-fin spine II shorter than or equal to III (11.0–18.4 vs. 13.0–20.2% SL; tip of anal-fin spine II broken in lectotype), relatively longer in juveniles, soft rayed portion of anal fin with anterior rays longest, posterior rays gradually shortening, posterior margin perpendicular to body axis or with slight posterior slant, anterior ray tips directly ventral to or forward of posterior tips, anterior tip of anal fin slightly rounded. Pectoral fins with ray 10 or 11 longest, extending to or slightly anterior to vent, fin-ray length 24.4–31.0 (25.5) % SL; fin-base width 8.4–10.4 (9.1) % SL; dorsalmost ray simple, ventral 5–10 simple, others branched in adults; all rays simple in three juveniles less than 90 mm (63.4–77.1 mm), the smallest examined. Pelvic fins extending about 50–90% of distance from pelvic-fin base to anal-fin origin, falling well short of vent, ray length 19.3–25.1 (22.8) % SL, spine length 51.7–76.0 (59.2) % ray length. All fin spines exhibit allometric growth, and juveniles have relatively longer spines. Caudal fin shallowly emarginate, length 20.0–28.1% SL (fin damaged in lectotype). Vent positioned below dorsal-fin spine 10–11, 7–21 (9.4) % HL from anal-fin origin.

Table 6

Factor loadings for principal component (PC) analysis of meristic characters of *Sebastes aleutianus* (rougheye rockfish) and *Sebastes melanostictus* (blackspotted rockfish) in which all characters were used for multivariate analyses.

	PC1	PC2	PC3
Dorsal–fin rays	0.5620	0.2917	–0.0630
Anal–fin rays	0.2956	0.5901	–0.5291
Pectoral–fin rays (left)	0.1390	–0.6710	–0.5828
Lateral–line pores (left)	0.4905	–0.1233	0.6118
Gill rakers	0.5804	–0.3181	–0.0470

Lateral body scales often with many (ca. 5–7) accessory scales in posterior field. Maxilla and underside of

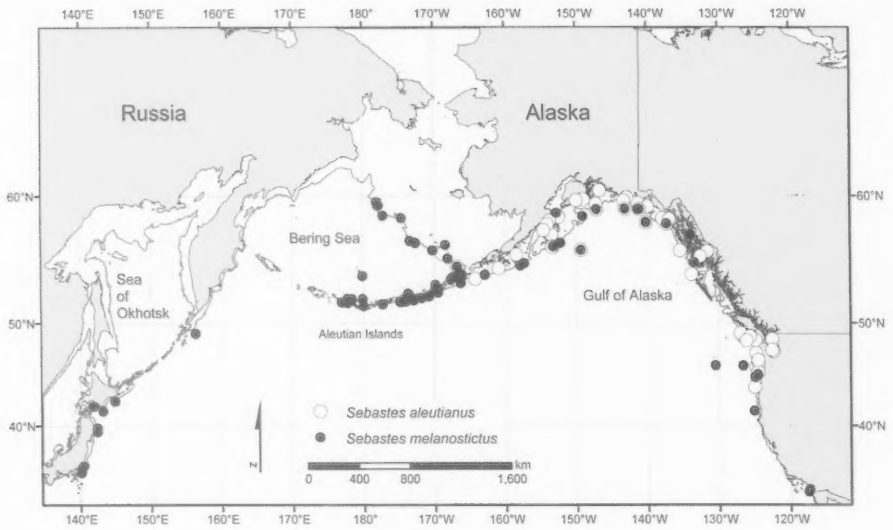

Figure 7

Distribution of *Sebastes aleutianus* (rougheye rockfish, open circle) and *Sebastes melanostictus* (blackspotted rockfish, closed circle) based on material examined. Closed circles superimposed on open circles represent collections of both species at the same locality. Each symbol may represent more than one specimen.

mandible completely scaled; suborbital region scaled; branchiostegal rays scaled.

Gill rakers relatively short, 3.4–6.3 (4.5) % SL (Fig. 5C), and slender on first arch; longest raker in joint between epi- and ceratobranchials; length of preceding rakers on upper arch and succeeding rakers on lower arch progressively shorter; rudiments absent. Extrinsic swimbladder muscle is Type I a–z of Hallacher (1974).

Body color in life pink, red, or reddish orange and darker brownish-red mottling is present in faint bands at and above the lateral line, often extending onto dorsal-fin base. Head light, with irregular shaped dark blotch at posterodorsal corner, other blotches often present on operculum between orbit and lower posterior margin. Orobuccal membranes pink to red, often with dark blotches; jaw membranes light, occasionally dusky. Spinous and soft dorsal fins uniformly pink to red, usually dark along fin margins, occasionally with small diffuse blotches near base of fin. Anal and caudal fins uniformly pink to red, dark along fin margin. Paired fins red, rays often with dark tips. Peritoneum gray to dusky, rarely white or black; stomach, pyloric caeca, and intestines pale. See Figures 1 and 2 and color figures in *S. aleutianus* species accounts of Love (2002; "juvenile" upper left), Kramer and O'Connell (1986, 1988, 1995, 2003; "juvenile"). Juveniles in life similar to adults in general body color, often with more distinct dark red to brown mottling. After preservation, reddish background color fading to light gray, yellowing with age. Dark areas remaining dark brown to black.

No sexual dimorphism is evident in morphometric or meristic characters. Largest specimen examined 555 mm (726 mm total length [TL], 710 mm fork length [FL]; FAKU 119293).

Distribution

The range of *Sebastes aleutianus,* based on material examined, extends from the eastern Aleutian Islands off Unalaska Island and the eastern Bering Sea at Pribilof Canyon at 55.7°N, south to southern Oregon at 43.9°N (Fig. 7). This distribution is nearly identical to that reported in the analyses of Hawkins et al. (2005, as *S. aleutianus*) and Gharrett et al. (2005, 2006, as "Type II"). Our material was collected at depths of 45 m to at least 439 m—a range that overlapped the depth distribution of *S. melanostictus* but which was typically shallower than the depth range of *S. melanostictus* (ANOVA, F=14.98, df=1, P=0.002; Fig. 8). No specimens of *S. aleutianus* are for certain from California, although at least one individual has been captured in northern California and was documented photographically. Catch data from AFSC surveys of the continental shelf and upper continental slope conducted from 1961 to 2001 indicate that neither *S. aleutianus* nor *S. melanostictus* (all identified as *Sebastes aleutianus*) was common in catches off California; only 61 specimens in 36 hauls were recorded for over 3600 tows of survey data (Fig. 9).

Etymology

The specific name *aleutianus* refers to the Aleutian Islands region. At the time of its original description (Jordan and Evermann, 1898), Kodiak Island was consid-

ered part of the geographic area now generally accepted to extend eastward only to Unimak Pass.

Remarks

The four syntypes of *Sebastodes aleutianus* were deposited at the USNM and CAS. The single USNM specimen (USNM 48800) was labeled "cotype" and is hereby designated as the lectotype of *Sebastes aleutianus*. Among the syntypes, this was the only specimen illustrated (Jordan and Gilbert, 1899, plate 48). The three specimens at CAS (SU 12928) are designated paralectotypes.

Individuals of *S. aleutianus* were apparently confused by Pallas (1814) as the "summer form" of *S. variabilis* (Pallas, 1814) (Jordan and Evermann, 1898), the dusky rockfish, one of the first species of *Sebastes* described from the Pacific Ocean—the first being its sister-species *S. ciliatus* (Tilesius, 1813), the dark rockfish (Kendall, 2000; Orr and Blackburn, 2004; Hyde and Vetter, 2007). Only a single specimen (MNHN 8670) is extant from the material Pallas examined for his description of *Perca variabilis*, and it has been designated the lectotype of *S. variabilis* (Orr and Blackburn, 2004). The only other specimens identified as *Perca variabilis* by Pallas were housed at ZMB and are missing, presumed to have been destroyed during the Second World War (Paepke and Fricke, 1992).

Evermann and Goldsborough (1907) described *Sebastodes swifti* from two juveniles collected in a bay of southeastern Alaska. Although the authors compared them to *S. crameri* (Jordan in Gilbert, 1897), the darkblotched rockfish, both specimens exhibited the diagnostic morphological characters and pale, unspot-

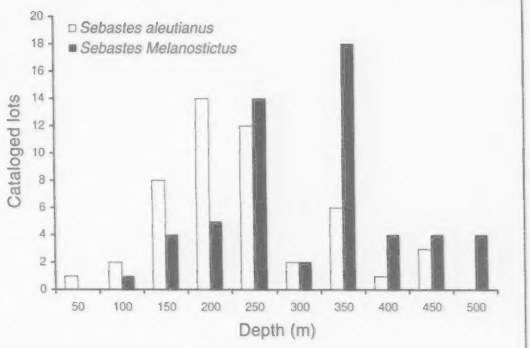

Figure 8

Depth (m) distribution of *Sebastes aleutianus* (rougheye rockfish, open bar) and *Sebastes melanostictus* (blackspotted rockfish, closed bar) based on material examined. Each bar represents numbers of catalogued museum lots, which may contain one or many specimens. *Sebastes melanostictus* is found at deeper depths than *S. aleutianus* (ANOVA, F=14.98, df=1, P=0.002).

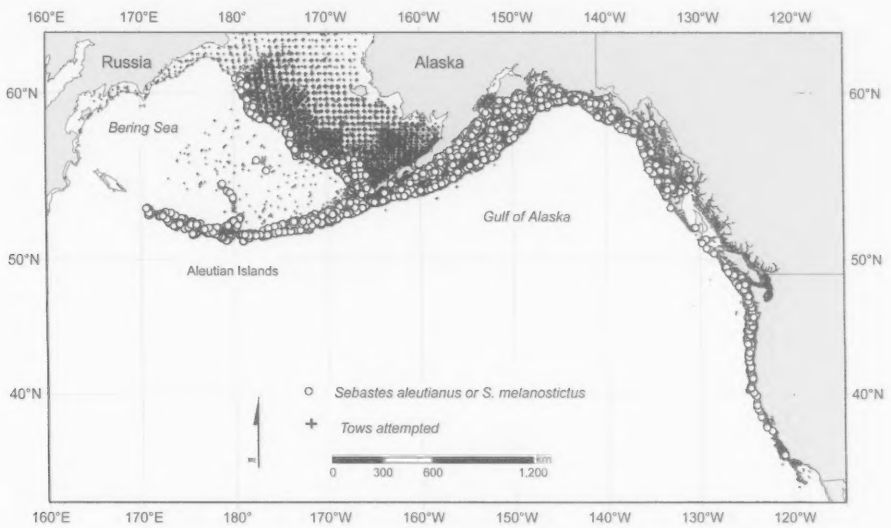

Figure 9

Distribution of the rougheye rockfish complex (open circle) in the eastern Pacific Ocean and Bering Sea based on National Marine Fisheries Service, Alaska Fisheries Science Center, survey data for the years 1961 to 2005. The rougheye rockfish complex includes *Sebastes aleutianus* and *S. melanostictus*, rougheye and blackspotted rockfish, which were recorded in 7758 tows. Although catch records from the northwestern Bering Sea indicated an absence of both species, species of *Sebastes* other than *S. alutus* (Pacific ocean perch) were routinely identified only to genus. Most symbols represent more than one tow and capture.

ted coloration of *S. aleutianus*, falling well within the variation of our material examined.

Sebastes melanostictus (Matsubara, 1934)
Blackspotted rockfish
Figures 3–8; Tables 1, 2, 4, 5

Sebastodes melanostictus Matsubara, 1934:206 (original description, one specimen: holotype FAKU 101043, male, 507 mm, "Prov. Kazusa").
Sebastodes kawaradae Matsubara, 1934:207 (original description, one specimen: FAKU 101042, male, 361.7 mm, Japan "off Miyako, near Morioka").
Sebastes melanostictus: Matsubara, 1943:209 (new combination).

Diagnosis

This species of *Sebastes* is distinguished from all other species, except *S. aleutianus*, in having eight pairs of head spines and two or more infraorbital spines. It is distinguished from *S. aleutianus* by the following combination of color patterns and morphological and genetic characteristics: body and spinous-dorsal fin spotted or, rarely, without spots, dusky overall with diffuse mottling on dorsum; dorsal-fin spine 1 longer, 1.0–1.8 times into orbit length, 5.9–9.6% SL (vs. 1.5–3.0 times into orbit length and 4.3–7.2% SL); dorsal-fin spine 4 longer, 12.0–18.4% SL (vs. 9.8–15.5% SL); gill rakers longer, 3.9–7.1% SL (vs.3.4–6.3% SL), and more numerous, 30–36 (vs. 29–34) (Table 2). Genetically diagnosed by a combination of the presence of protein variants *ACP*46*, either *IDDH*500* or **750* or both, either *PGM-2*83* or **74* or both, and *XO*109* (Hawkins et al., 2005); homozygosity at microsatellite allele *μSma6*177* (Gharrett et al., 2005, 2006); and mitochondrial haplotype A of Gharrett et al. (2005, 2006).

Description

D XII–XIV (XIII), 12–15 (14); A III, 7–8 (7); P1 17–19 (18), 5–10 (7) simple; lateral-line pores 30–36 (31), scales 40–56; infraorbital spines 2–12 (5); gill rakers 30–36 (8–11 + 21–26) (33: 9+24); vertebrae 26–27 (10 + 16–17) (27, Matsubara, 1934); pyloric caeca 9–12. Meristic frequency and statistical data are presented in Tables 1, 2, 4, and 5.

Body robust, depth at pelvic-fin base 31.3–40.1 (37.5) % SL; relatively slender at anal-fin origin 22.8–30.5 (28.7) % SL, profile of dorsal margin of head gently sloping from dorsal-fin origin to snout, dorsal rim of orbit included in lateral margin of frontals. Mouth large, with posterior end of maxilla extending between pupil and posterior rim of orbit to just beyond the posterior rim of the orbit; maxilla length 17.2–21.5 (20.0) % SL; symphyseal knob moderately pronounced with blunt tip; lower jaw length 21.6–27.1 (25.9) % SL; mandibular pores large to moderate in size. Cranial spines strong, often rough. Nasal, preocular, supraocular, postocular, tympanic, coronal, parietal, and supratemporal spines invariably present; all major head spines strong, except for the coronal spines, which are weak; supraocular and postocular spines and parietal ridge often rough; sphenotic spines obsolete, occasionally moderate in size. Interorbital region wide, 5.6–9.2 (8.8) % SL, flat to slightly concave; frontal ridges between orbits obsolete to moderate; parietal ridges strong, and area between ridges slightly convex or flat. Preopercular spines 5, directed posteroventrally; opercular spines 2, upper spine directed posteriorly, lower spine directed posteriorly and slightly ventrally. Posttemporal and supracleithral spines present. Ventral margin of lachrymal with two rounded lobes, both anterior and posterior often with a single small posteriorly directed spine, posterior occasionally with as many as 4 or 5 small spines forming a serrate margin. Infraorbital spines small, 2–12 (5): 0–4 (2) spines on first infraorbital, 0–6 (2) on second, and 0–4 (1) on third (7 on third infraorbital of the left side of one anomalous specimen, UW 116497); dorsal margin of opercle nearly horizontal; lower margin of gill cover with two small spines, produced by the posteroventral tip of interopercle and anteroventral tip of subopercle.

Dorsal-fin origin above anterodorsal portion of gill slit; dorsal fin continuous, gradually increasing in height from spine I (5.9–9.6% SL, 1.5–3.0 times into orbit length; Fig. 5A) to spine IV (12.0–18.4% SL; Fig. 5B; tips of dorsal-fin spines broken in holotype) and decreasing in height to spine XII; spine XIII much larger, forming anterior support of dorsal-fin rays; membranes of spinous dorsal fin moderately incised, less so posteriorly; soft dorsal fin with anterior rays longest, posterior rays gradually shortening. Anal-fin spine II slightly shorter than or equal to III (11.8–19.6 vs. 12.2–21.3% SL; tips of anal-fin spines broken in holotype), relatively longer in juveniles, soft rayed portion of anal fin with anterior rays longest, posterior rays gradually shortening, posterior margin perpendicular to body axis or with slight posterior slant, anterior ray tips directly ventral to or forward of posterior tips, anterior tip of anal fin slightly rounded. Pectoral fins with ray 10 or 11 longest, extending to or slightly anterior to vent, fin-ray length 22.0–30.0 (27.9) % SL; fin-base width 8.2–10.4 (10.2) % SL; dorsalmost ray simple, ventral 5–10 simple, others branched. Pelvic fins extending about 50–90% of distance from pelvic-fin base to anal-fin origin, falling well short of vent, ray length 18.8–25.0 (23.1) % SL, spine length 55.1–72.9 (58.6) % ray length. All fin spines exhibiting allometric growth, with juveniles having relatively longer spines. Caudal fin shallowly emarginate, length 19.2–25.2 (22.2) % SL. Vent positioned below dorsal-fin spine 10–11, 7–21 (13.8) % HL from anal-fin origin.

Lateral body scales often with many (ca. 5–7) accessory scales in posterior field. Maxilla and underside of mandible completely scaled; suborbital region scaled; branchiostegal rays scaled.

Gill rakers long, 3.9–7.1 (6.6) % SL, and slender on first arch; longest raker in joint between epi- and ceratobranchials; length of preceding rakers on upper arch and succeeding rakers on lower arch progressively

shorter; rudiments absent. Extrinsic swimbladder muscle Type I a–z of Hallacher (1974).

Body color in life light to dark, often heavily mottled. Light specimens pink to red and with greenish-black spotting below and above the lateral line and on dorsum between lateral line and base of dorsal fins, extending onto spinous and often soft dorsal-fin membranes, occasionally with a yellow wash. Dark specimens with black to greenish wash over red to pink background color, heavily mottled over entire body and spotted at and above the lateral line; heavy dark mottling often obscuring spotting. Body rarely dusky overall but having diffuse mottling, without spotting. Three white blotches often present along base of dorsal fin: anterior blotch at base of dorsal-fin spines 2–4, middle blotch at base of spines 11–12 and anteriormost rays, posterior blotch at base of posteriormost dorsal-fin rays and along anterior portion of caudal peduncle. Head light or dark, with three vague dusky bands often extending from side to side: one across region of nares, a second just posterior to orbits, and a third at posterior half of parietal spines. Head otherwise similar to body coloration, with irregular-shaped dark blotch at posterodorsal corner, other blotches often present on operculum between orbit and lower posterior margin. Iris golden to brassy yellow. Orobuccal membranes blotched or dark on pink to red background; jaw membranes dusky or dark. Spinous and soft dorsal fins variously spotted, on a pink to red or dusky background, usually dark along fin margins. On spinous dorsal fin, spots commonly in two general areas: anteriorly between spines 1–3 and posteriorly between spines 5–11. Anal and caudal fins uniformly pink to red, dark along fin margin. Paired fins red, rays often with dark tips. Peritoneum dusky to dark, rarely light or black; stomach, pyloric caeca, and intestines pale. See Figures 3 and 4 and the color figures identified as *S. aleutianus* or rougheye rockfish of Kanayama and Kitagawa (1982), Amaoka (1984), Kessler (1985), Orr et al. (1998, 2000), Love (2002, lower left and upper right), and Gharrett et al. (2006, bottom dark form). Juveniles in life similar to adults in general body color, often with more profuse spotting. After preservation, reddish background color fading to light gray, yellowing with age. Spotting on dorsal fins and dark areas on body remaining dark brown to black.

No sexual dimorphism in morphometric or meristic characters is evident. Largest specimen examined 539 mm (690 mm TL, 660 mm FL; FAKU 119236).

Distribution and natural history

The range of *Sebastes melanostictus* based on material examined extends from the Pacific coast of Japan, at about 35°N, north through the Kuril Islands, Aleutian Islands, and the Bering Sea to 60.5°N, and south to southern California on Coronado Bank, at 32.6°N (Fig. 7). Our material extends the range of *S. melanostictus*, previously reported by Hawkins et al. (2005, as *S.* sp. cf. *aleutianus*) and Gharrett et al. (2005, 2006, as "Type I"), to the western Pacific south of the Bering Sea to central Japan and in the eastern Pacific to southern California.

Collected at depths from 84 to at least 490 m, *S. melanostictus* is more common at deeper depths than *S. aleutianus* among material examined (ANOVA, F=14.98, df=1, P=0.002; Fig. 8). In the Kuril Islands and southeastern Kamchatka, areas where only *S. melanostictus* is known, it is found from 200 to 650 m and is most abundant between 350 and 450 m depth (Orlov, 2005). Two specimens (UW 116884 and UW 116885) were recently captured in midwaters of the Gulf of Alaska at a maximum gear depth of 500–1000 m over 1800–1900 m of bottom depth. Nearly 150 midwater tows during surveys conducted by AFSC captured *S. aleutianus* or *S. melanostictus* (identified as *S. aleutianus*) at depths of 15–3355 m above the bottom. All were captured north of Washington waters in Alaska and Canada (Fig. 9).

Etymology

The specific name *melanostictus* is derived from the Greek μέλας for "black" and στικτος for "spot" referring to the spotted body coloration.

Remarks

A surprising conclusion of our work is that *S. kawaradae* is synonymous with *S. melanostictus* rather than *S. aleutianus*. As described by Matsubara (1934) and verified in our examination, the body of the holotype of *S. kawaradae* is dusky and lacks the spotting typically present in *S. melanostictus*. Although this color pattern is uncommon in *S. melanostictus*, it is a pattern not found in the material examined and identified as *S. aleutianus* by either genetic or morphological analysis in this revision.

Matsubara (1934) published his original description of both species in the same paper; *S. melanostictus* was followed immediately by *S. kawaradae*, each based only on the holotype. He distinguished the two by several characters, including differences in body color, describing *S. kawaradae* in formalin as dark brown without spots as opposed to *S. melanostictus*, grayish with spots. Other characters included peritoneum silvery rather than black; orbit length longer than either the interorbital width or snout length versus equal to interorbital width or shorter than snout length; infraorbital spines 5 rather than 4; and lateral line pores 34 rather than 31. These characters all fall within the range of our material of *S. melanostictus*.

Later, Matsubara (1943) described additional aspects of the holotype of *S. kawaradae*, including the structure of the infraorbitals and air bladder and counts of vertebrae, and compared the holotype with *S. aleutianus*, from which he distinguished it on the basis of three characters: a higher gill-raker count of 32, narrower interorbital space, and longer second anal-fin spine. Matsubara's (1943) gill raker count of 24 for *S. aleutianus* was apparently repeated from

Jordan and Evermann (1898), which is an error because the four syntypes of *S. aleutianus* have a range of 30–32 gill rakers. In our material, the gill-raker count of 32 lies within the broad range of overlap in numbers of gill rakers in *S. aleutianus* and *S. melanostictus* (Table 5). Although the length of the second anal-fin spine is longer than in all other *S. aleutianus* of similar size, it is similar in size to the second anal-fin spine of all *S. melanostictus*. Interorbital widths of *S. aleutianus* and *S. melanostictus* differ slightly, and *S. kawaradae* has the wider interorbital of similar size *S. melanostictus*. Based on material examined from throughout the range of the species, all other morphometric and meristic data support the conclusion that *S. kawaradae* is a synonym of *S. melanostictus*.

Examining specimens from the northeastern Gulf of Alaska, Gharrett et al. (2006) distinguished by morphological techniques more than 94% of specimens that had been earlier identified by genetic analyses. They found concordant results between allozyme data (Seeb, 1986; Hawkins et al., 2005) and DNA markers (Gharrett et al., 2005). The results of our work, extending the morphological examination across nearly the entire geographic range of both species, are very similar to theirs with a slightly higher degree of resolution. Detailed diagnostic color differences, as well as a combination of morphological characters, distinguish the two species (the dark "Type I" and light "Type II" of Gharrett et al., 2006). Most importantly, the persisting confusion between the light and dark "Types" is resolved. Most specimens of *S. melanostictus* ("Type I") display an overall darker coloration than *S. aleutianus* ("Type II"), but this "darker" coloration may range from a dusky or blotched color pattern over the body to a few dark spots on a light background—a pattern that would presumably be called "light" by field biologists. In contrast, *S. aleutianus* is always pale and would invariably be described as light in the field.

The length of the first dorsal-fin spine is of particular importance in distinguishing between the two species. Gharrett et al. (2006) did not measure the first dorsal-fin spine length but found the "longest dorsal-fin spine" to differ significantly between the two species. Among our material, the length of the fourth dorsal-fin spine, which is most often the longest spine, is significantly different but does not differ consistently enough to be useful as a field character. In contrast, especially in field identifications when body color was equivocal, the length of the first dorsal-fin spine was particularly useful. Its heavy weighting in the discriminant function equation underlines its importance.

Evidence of a low-level of hybridization between *S. aleutianus* and *S. melanostictus* has been found from analyses with allozyme data (Hawkins et al., 2005) and DNA markers (Gharrett et al., 2005). Both Hawkins et al. (2005) and Gharrett et al. (2005) found <1% of possible hybrids in their large sample sizes. Although a few specimens have uncertain genotypes in our allozyme analysis, no individuals were determined conclusively to be hybrids with concepts of hybrid intermediacy, because the range of variation among all morphological characters overlapped to some degree between the two species. However, one individual (UW 116878, 236.0 mm) from the northeastern Gulf of Alaska identified as *S. aleutianus* because of color and general morphological features had the genetic signature of a possible hybrid and was also placed in the overlapping region of the PCA. It was captured with longline gear together with specimens of *S. melanostictus* and other specimens of *S. aleutianus* at an intermediate depth of 300–400 m.

Phenotypically, two species in the eastern North Pacific region are similar and often difficult to distinguish from *S. aleutianus* and *S. melanostictus*. *Sebastes borealis* is the deepest dwelling species of the genus and, like *S. aleutianus* and *S. melanostictus*, is a robust red rockfish. Until the work of Tsuyuki and Westrheim (1970) and Barsukov (1970), it was treated as *S. aleutianus* or *S. melanostomus*. It also may have up to two infraorbital spines and similar cranial spines, although the coronal spine is typically absent. *Sebastes borealis* is uniform reddish-pink, unlike *S. melanostictus*, which invariably has spots on its fins or body and is generally darker in color. At larger sizes, above about 40 cm TL, *S. borealis* can be distinguished by the length of its gill rakers, which are shorter, ca. 5–15% head length, and fewer (27–31) than the longer, ca. 10–20 % HL, and more numerous (29–36) gill rakers of both *S. aleutianus* and *S. melanostictus*.

Sebastes melanostomus is readily distinguished underwater by color (Butler and Love, 2002), exhibiting distinct blotches of red and white, unlike either the uniformly pale *S. aleutianus* or distinctly spotted *S. melanostictus*. When captured and brought to the surface, it is similar to *S. aleutianus* in coloration, having a reddish-pink background coloration and some mottling, especially around the head and on the branchiostegal membranes. It lacks the spotting on the body and fins of *S. melanostictus* and may be distinguished from both species by having only 1 or 2 infraorbital spines and usual absence of the coronal spine.

Four species of western Pacific *Sebastes* are considered to be morphologically similar to *S. aleutianus* and *S. melanostictus*: *Sebastes matsubarai*[1] Hilgendorf (1880), akôdai; *S. baramenuke* (Wakiya, 1917), baramenuke; *S. flammeus* (Jordan and Starks, 1904), sankômenuke; and *S. iracundus* (Jordan and Starks, 1904), ôsaga. All are relatively large and robust reddish rockfishes that live at greater depths than do most other species. As a group they, including *S. melanostictus*, are referred

[1] As noted in editions of Eschmeyer (1998), the name *Sebastes matsubarae* Hilgendorf requires emendation to *Sebastes matsubarai* Hilgendorf because it was named for the male ichthyologist Shin-Nosuke Matsubara (Hilgendorf, 1880: footnote p. 14), a professor of the University of Tokyo and leader of an expedition in the waters around Japan in the mid-1800s, who also served as a translator for F. M. Hilgendorf (Abe, 1986).

to as "menuke" in Japanese, meaning "exploding eye" because of the bulging eyeballs seen in fishes brought to the surface after being captured at depth. Except for *S. matsubarai*, each of these is easily distinguished from both *S. melanostictus* and *S. aleutianus* by the absence of infraorbital spines. In *S. matsubarai*, two spines are present but each is strong and invariably present in the same position: one on the lachrymal and one on the base of the suborbital stay of infraorbital 3. In addition, the lachrymal has two or three strong spines on its ventral margin, in contrast with the two typically rounded or flattened lobes, the second with up to four small spines in *S. melanostictus* and *S. aleutianus*.

Sebastes flammeus and *S. iracundus* are very similar to one another and were considered synonymous by Balanov et al. (2004). However, both Kai et al. (2003) and Hyde and Vetter (2007) found differences in genetic sequences between the two species, providing additional evidence for their validity as separate species. They are morphologically most similar to *S. borealis*, with which they appear to be closely related (Hyde and Vetter, 2007). Both lack infraorbital spines and have reduced cranial spines that lack coronal and nuchal spines.

A recent comprehensive phylogenetic analysis of *Sebastes*, based on molecular data conducted by Hyde and Vetter (2007), revealed notable differences as well as similarities between ideas of relationships based on overall morphological similarity and detailed genetic analyses. Based on maximum parsimony and Bayesian phylogenetic hypotheses, the subgenus *Zalopyr* was placed in a basal position within the tree and encompassed *S. aleutianus* and *S. melanostictus*, as well as the western Pacific species *S. glaucus* Hilgendorf (1880), gray rockfish; *S. steindachneri* Hilgendorf (1880), yanaginomai; *S. minor* Barsukov (1972), akagaya; and *S. owstoni* (Jordan and Thompson, 1914), hatsume. The clade comprising *Sebastes aleutianus* and *S. melanostictus* was placed in a terminal position in relation to the western Pacific species. All other species of *Zalopyr* of Hyde and Vetter (2007) are readily distinguished from *S. aleutianus* and *S. melanostictus* by the absence of infraorbital spines and reduced or obsolete cranial spines. Only *S. aleutianus* and *S. melanostictus* are deeper-water species—both species commonly occurring below 300 m and at least as deep as 500 m; all other species within *Zalopyr* are more shallow-water species recorded to a maximum depth of 300 m.

The purpose of naming elements of phylogenetic trees is to communicate information on evolutionary relationships, primarily the existence of monophyletic groups. The subgenus *Zalopyr* Jordan and Evermann (1898) was originally erected for *Sebastes aleutianus* (the type species of the subgenus) and *S. atrovirens* (Jordan and Gilbert, 1880) (Kendall, 2000). Although *Zalopyr* of Hyde and Vetter (2007) comprised a monophyletic group, the inclusion of any species other than *S. aleutianus* and *S. melanostictus* on the basis of molecular evidence alone is premature, particularly because only the clade including *S. aleutianus* and *S. melanostictus* may be readily diagnosed morphologically. The name *Zalopyr* should be restricted to the terminal clade of *S. aleutianus* and *S. melanostictus*, a subgenus diagnosed by the combination of two character states: the presence of 2–10 infraorbital spines and 8 pairs of major cranial spines. To avoid naming a paraphyletic group, the species in the more basal portion of the clade should be 1) removed from *Zalopyr* and placed in their own subgenera, 2) the name *Emmelas* Jordan and Evermann (1898) applied to the basalmost member, *S. glaucus*, and 3) the name *Hatumeus* Matsubara (1943), previously used for the monotypic subgenus comprising *S. owstoni*, applied to the western Pacific clade that also includes *S. steindachneri* and *S. minor*.

This phylogenetic hypothesis indicates the derivation of *S. melanostictus* and *S. aleutianus* from a western North Pacific and shallow-water ancestor that dispersed into deeper waters of the eastern North Pacific region. Their divergence from other members of the clade has been estimated to have occurred at over 4.5 million years ago (Hyde and Vetter, 2007) and from one another at about several hundred thousand to just over one million years ago (Gharrett et al., 2005; Hyde and Vetter, 2007).

The zoogeographic pattern exhibited by *Sebastes aleutianus* and *S. melanostictus* is similar to several other species pairs found in waters of Alaska, including *Sebastolobus alascanus* Bean (1890) and *S. altivelis* Gilbert (1896), the long- and shortspine thornyheads; *Lepidopsetta polyxystra* Orr and Matarese (2000) and *L. bilineata* (Ayres, 1855), the northern and southern rock soles; and *Lycodes palearis* Gilbert (1896) and *L. brevipes* Bean (1890), the wattled and shortfin eelpouts (Mecklenburg et al., 2002). In each of these pairs, one species ranges across the North Pacific region from the Kuril Islands in the west to California in the east. The other species is an eastern North Pacific species, ranging more or less into the Bering Sea and ending to the west in the Aleutian Islands at Samalga Pass, the western extent of the ancient and now fragmented Alaska Peninsula. Recent studies across a wide spectrum of organisms, from whales and seabirds to fishes and zooplankton, across the Aleutian Islands have found differences in the nature of oceanographic ecosystems on either side of Samalga Pass: to the east the environment is more like that of the continental shelf, whereas to the west it is more oceanic (Hunt and Stabeno, 2005). Although many of the distribution patterns may be related to geologically recent ecological factors, a deeper vicariant event may have been responsible for affecting species distributions in the region (Logerwell et al., 2005)

Fisheries for rockfish on the U.S. west coast are presently managed by species, geographic region, and habitat (primarily depth) parameters. Although overlapping broadly in distribution, *S. aleutianus* is typically found at shallower depths than *S. melanostictus* (Fig. 8; Hawkins et al., 2005; Gharrett et al., 2005, 2006), and, under present management regimes, *S. melanostictus* may be fished more heavily because the major part of its distribution lies outside the more shallow restricted ar-

eas. Recent preliminary reports from fisheries observers off the Washington and Oregon coasts have indicated a predominance of *S. melanostictus* in catches—catches that because of fisheries restrictions were at depths of 457 m (250 fm) or greater. As more information on the biology of both species is gathered following their future identification in surveys and fisheries, a better understanding of management needs and strategies is certain to emerge.

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Appendix

Material examined

Sebastes aleutianus 131 specimens examined, 63.4–555.2 mm, including the types listed above. **Bering Sea:** UW 113740, 1 (285 mm), 55.7091°N, 168.8126°W, 225 m depth, 21 July 2004, FV *Northwest Explorer*, J.W. Orr; FAKU 119255, 1 (277.3 mm), 54.6887°N, 166.675°W, 330 m depth; FAKU 119293, 1 (555.2 mm), 54.6887°N, 166.675°W, 330 m depth. **Aleutian Islands:** UW 49327, 6 (123.7–272.8 mm), 53.7241°N, 166.8921°W, 188 m depth, 23 May 2000, FV *Dominator*, J.W. Orr; UW 114740, 3 (213–230 mm), 53.7457°N, 166.9795°W, 185 m depth, 23 May 2000, FV *Dominator*, J.W. Orr; UW 114779, 2 (254–259 mm), 58.4864°N, 152.3621°W, 154 m depth, 4 July 2001, FV *Vesteraalen*, J.W. Orr; FAKU 131033, 1 (239.9 mm), 53.79852°N, 166.3926°W, 249 m depth, 25 May 1996, FV *Golden Dawn*, J.W. Orr. **Gulf of Alaska:** KU 28070, 1 (215.8 mm), 59.6317°N, 143.1794°W, 250 m depth, 10 July 1999, FV *Morning Star*, K. Shaw; UW 46609, 2 (176.5–163.9 mm), 59.8329°N, 142.7660°W, 189 m depth, 19 July 1996; UW 45729, 8 (197.2–221.8 mm), 58.3142°N, 136.8035°W, 77 m depth, 23 July 1996, FV *Dominator*, J.W. Orr; UW 111746, 2 (460–470 mm), 56.279°N, 153.1482°W, 349 m depth, 23 March 2002, RV *Miller Freeman*, C. Wilson; UW 112700, 3 (147–265 mm), 57.9453°N, 153.9643°W, 197 m depth, 28 June 2003, FV *Gladiator*, J.W. Orr; UW 112646, 3 (240.2–345 mm), 59.4547°N, 141.1970°W, 300 m depth, 31 March 2003, RV *Miller Freeman*, C. Wilson; UW 112318, 1 (370 mm), 53.7252°N, 164.1641°W, 314 m depth, 26 May 2001, FV *Morning Star*, J.W. Orr; UW 113744, 1 (348 mm), 58.1717°N, 153.6359°W, 194 m depth, 4 July 2003, FV *Gladiator*, J.W. Orr; UW 113746, 1 (290 mm), 58.21164°N, 153.6442°W, 185 m depth, 4 July 2003, FV *Gladiator*, J.W. Orr; UW 113715, 1 (105 mm), 58.4728°N, 152.3812°W, 148 m depth, 10 July 2003, FV *Gladiator*, J.W. Orr; UW 113748, 1 (365 mm), 56.2331°N, 153.3164°W, 240 m depth, 24 June 2001, FV *Vesteraalen*, J.W. Orr; UW 114772, 6 (147–345 mm), 58.3627°N, 137.8006°W, 178 m depth, 17 July 1999, FV *Vesteraalen*; UW 114741, 1 (120 mm), 55.6373°N, 158.3360°W, 137 m depth, 9 June 2001, FV *Vesteraalen*, J.W. Orr; UW 114773, 1 (392 mm), 59.6434°N, 149.8903°W, 212 m depth, 29 June 1999, FV *Morning Star*; HUMZ 34389, 1 (343.2 mm), 55.9667°N, 135.3°W, 6 July 1969; HUMZ 34523, 1 (369.6 mm), 55.9667°N, 135.3°W, 6 July 1969; HUMZ 34513, 1 (420.5 mm), 54.0833°N, 133.7°W; HUMZ 67970, 1 (366.2 mm), 54.5833°N, 160.8667°W; HUMZ 102217, 1 (355.8 mm), 56°N, 149.25°W, 333 m depth, 15 August 1984; HUMZ 102215, 1 (358.7 mm), 56°N, 149.25°W, 333 m depth, 15 August 1984; UW 116468*, 3 (120.2–138.6 mm), 59.6918°N, 148.8569°W, 180 m depth, 28 July 2005, FV *Northwest Explorer*, J. W. Orr; UW 116485*, 4 (227–292 mm), 57.1615°N, 153.3414°W, 117 m depth, 2 August 2005, FV *Northwest Explorer*, J. W. Orr; UW 110840*, 10 (260–385 mm), 59.0333°N, 141.0167°W, 300–400 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 110841*, 4 (255–390 mm), 59.05°N, 143.0167°W, 300–600 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 110438*, 3 (261–396 mm), 59.0333°N, 141.0167°W, 300–400 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 116880*, 2(297–345 mm), 59.0167°N, 147.033°W, 300–400 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 116878*, 2 (236–262.9 mm), 58.0667°N, 140.067°W, 300–400 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 114762, 4 (148–204 mm), 59.4046°N, 147.0968°W, 202 m depth, 7 July 1999, FV *Vesteraalen*; UW 115760, 4 (103.7–136.3 mm), 59.21094°N, 139.8543°W, 138 m depth, 15 July 1999, FV *Vesteraalen*, J. W. Orr; UW 49125, 1 (135 mm), Prince William Sound, Port Etches, 60.3428°N, 146.5956°W, 30 May 1990, 45 m depth, FV *St. George*, A. M. Shedlock; UW 115762, 2 (128.7–196 mm), 57.9752°N, 137.1904°W, 282 m depth, 17 July 1999, J. W. Orr. **Canada:** CAS 15288, 1 (372 mm), off Vancouver I., La Perouse Bank, 48.38°N, 126.0883°W, 307 m depth, 11 September 1972, RV *G.B. Reed*, T. Iwamoto. **Washington:** UW 2735, 4 (63.4–79 mm), Puget Sound; UW 48307, 1(248 mm), Puget Sound, off West Point; CAS-SU 21303, 1 (77.1 mm), Puget Sound, San Juan I., E.C Starks; UW 20990, 1 (144 mm), Puget Sound, Pt. Pulley, 200 m depth, 15 February 1983; UW 20714, 1 (139.9 mm), Puget Sound, West Point, RV *Commando*, 238 m depth, 2 November 1971, A. C. DeLacy; UW 25963, 1 (138.7 mm), Puget Sound, off Saltwater Beach Park, 200 m depth; UW 49322, 1 (90.5 mm), 45.7307°N, 124.4742°W, 177 m depth, 31 July 2001, FV *Frosti*; UW 15671, 1 (217 mm), Cape Flattery, 7 October 1958; UW 114739*, 6 (201–273 mm), 48.7462°N, 125.5771°W, 152 m depth, 5 August 1998, FV *Vesteraalen*; UW 114774*, 1 (382 mm), 48.7540°N, 126.5163°W, 421 m depth, 3 August 1998, FV *Vesteraalen*; UW 114775*, 1 (340 mm), 49.0582°N, 126.9109°W, 439 m depth, 7 August 1998, FV *Vesteraalen*; UW 114771*, 3 (108.5–243 mm), 48.7640°N, 125.5406°W, 138 m depth, 5 August 1998, FV *Vesteraalen*; UW 114778*, 2 (163–193

mm), 47.216°N, 124.746°W, 125 m depth, 1 August 1998, FV *Vesteraalen*; UW 114767*, 1 (201 mm), 46.5476°N, 124.4576°W, 125 m depth, 20 July 1998, FV *Vesteraalen*; UW 114768*, 1 (193 mm), 46.54758°N, 124.4576°W, 125 m depth, 10 July 1998, FV *Vesteraalen*, J. W. Orr; UW 44200, 1 (375 mm), 48.35°N, 124.8667°W, 165 m depth, 12 September 1992, FV *Pacific Orion*, J. Firehammer; UW 44201, 1 (345 mm), 4.35°N, 124.8667°W, 293 m, FV *Island Enterprise*, F. Smith. **Oregon:** CAS 40415, 1 (270 mm), 45.4217°N, 124.45°W, 296 m depth, 28 August 1977, RV *Profesor Siedlecki*; UW 114738*, 3 (350–430 mm), 45.9042°N, 124.7654°W, 411 m depth, 19 July 1998, FV *Vesteraalen*; UW 114776*, 3 (170–265 mm), 46.2407°N, 124.4106°W, 242 m depth, 19 July 1998, FV *Vesteraalen*; UW 43415, 1 (377.5 mm), 43.9174°N, 124.9323°W, 387 m depth, 6 July 1998, FV *Vesteraalen*; UW 114037, 1 (165 mm), 44.7809°N, 124.514°W, 175 m depth, 23 July 1995, FV *Vesteraalen*.

Sebastes melanostictus 204 specimens examined, 95.5–539 mm, including the types listed above. **Japan:** FAKU 5760, 1 (365 mm), Japan, Akkeshi, Hokkaido Prefecture; FAKU 5769, 1(340 mm), Japan, Akkeshi, Hokkaido Prefecture; FAKU 5771, 1 (366.3 mm), Japan, Akkeshi, Hokkaido Prefecture; FAKU 5774, 1 (395 mm), Japan, Akkeshi, Hokkaido Prefecture; FAKU 5775, 1 (325 mm), Japan, Akkeshi, Hokkaido Prefecture; FAKU 81608, 1 (367.7 mm), Japan, off Miyako, Iwate Prefecture; HUMZ 97600, 1 (447.9 mm), Japan, Kushiro Fish Market, 30 miles SE of Cape Erimo; HUMZ 92733, 1 (441.9 mm), Kamaishi fish market, Iwate Prefecture; HUMZ 92746, 1 (419.9 mm), Kamaishi fish market, Iwate Prefecture; HUMZ 103024, 1 (441.6 mm), off Muroran. **Kuril Islands:** HUMZ 134873, 1 (407.3 mm), 49.0167°N, 156.4283°E, 159 m depth, 9 October 1994. **Bering Sea:** UW 46065, 1 (189.5 mm), 56.6981°N, 173.2926°W, 311 m depth, 17 July 2000, FV *Morning Star*, J. W. Orr; UW 111549, 1 (410 mm), 59.3031°N, 177.6877°W, 335 m depth, 14 July 2000, FV *Morning Star*, J. W. Orr; UW 111548, 1 (352.3 mm), 58.5697°N, 177.0833°W, 462 m depth, 16 July 2000, FV *Morning Star*, J. W. Orr; UW 49328, 1 (118 mm), 55.9686°N, 170.1104°W, 313 m depth, 6 June 2002, FV *Morning Star*, J. W. Orr; UW 113851, 3 (155.8–182.7 mm), 56.3865°N, 168.3234°W, 137 m depth, 27 July 2003, FV *Aldebaran*, J. R. Hoff; FAKU 119257, 1 (322.6 mm), 55.3308°N, 167.9962°W, 360 m depth; HUMZ 93691, 1 (368.8 mm), Bering Sea, 59.53°N, 177.97°W, 6 August 1981; HUMZ 93826, 1 (498.1 mm), Bering Sea, 56.54°N, 172.52°W, 18 August 1981; UW 113902, 1 (355 mm), 58.3978°N, 174.4882°W, 340 m depth, 27 June 2004, FV *Northwest Explorer*, D. E. Stevenson. **Aleutian Islands:** UW 48464, 1 (249.5 mm), 53.2207°N, 169.7395°W, 328 m depth, 2 June 2002, FV *Morning Star*, J. W. Orr; UW 49319, 4 (95.5–106.2 mm), 53.8149°N, 167.2937°W, 335 m depth, 26 May 2002, FV *Morning Star*, J. W. Orr; UW 49334, 1 (115 mm), 52.8973°N, 169.5051°W, 240 m depth, 2 June 2002, FV *Morning Star*, J. W. Orr; UW 112634, 1 (101.6 mm), 51.6083°N, 177.18°W, 224 m depth, 4 July 2002, FV *Sea Storm*, J. W. Orr; UW 112317, 1 (410 mm), 52.0601°N, 177.8333°E, 244 m depth, 29 July 1997, FV *Vesteraalen*, J. W. Orr; UW 114733, 4 (195–335 mm), 53.3605°N, 166.162°W, 315 m depth, 23 May 2001, FV *Morning Star*, J. W. Orr; UW 114769, 1 (415 mm), 53.6741°N, 167.5344°W, 309 m depth, 24 May 2000, FV *Dominator*, J. W. Orr; UW 112319, 1 (335 mm), 52.2933°N, 173.3717°W, 360 m depth, 3 June 2000, FV *Dominator*, J. W. Orr; UW 43296, 1 (356 mm), 52.0403°N, 178.5132°E, 389 m depth, 20 July 1994, FV *Pacific Knight*, R. R. Lauth; UW 43456, 1 (326.4 mm), 52.0601°N, 177.8333°E, 244 m depth, 29 July 1997, FV *Vesteraalen*, J. W. Orr; UW 114734, 1(269 mm), 52.5036°N, 173.5548°W, 238 m depth, 2 June 2000, FV *Dominator*, J.W. Orr; UW 114736, 1 (252 mm), 51.8025°N, 174.7188°W, 315 m depth, 7 July 1997, FV *Dominator*, R. R. Lauth; UW 114737, 3 (128–271 mm), 52.5987°N, 169.3605°W, 237 m depth, 20 May 2001, FV *Morning Star*, J. W. Orr; UW 114770, 1 (355 mm), 54.1176°N, 166.2599°W, 91 m depth, 22 May 2000, FV *Dominator*, J.W. Orr; UW 45528, 2 (243.8–247.6 mm), 52.7156°N, 169.8153°W, 148 m depth, FV *Golden Dawn*, J. W. Orr; UW 44202, 3 (305–370 mm), 51.7167°N, 178.3°W, 86 m depth, 7 July 1992, FV *Pavlof*, S. Sloane; FAKU 131030, 1 (268 mm), 54.0417°N, 162.7699°W, 193 m depth, 30 May 1996, FV *Golden Dawn*, J. W. Orr; FAKU 131031, 1 (316.0 mm), 54.0417°N, 162.7699°W, 193 m depth, 30 May 1996, FV *Golden Dawn*, J. W. Orr; FAKU 131032, 1 (343.5 mm), 54.0417°N, 162.7699°W, 193 m depth, 30 May 1996, FV *Golden Dawn*, J. W. Orr; FAKU 119236, 1 (539 mm), 51.7667°N, 177.0333°E, 100–885 m depth; FAKU 119245, 1 (373.7 mm), 54°N, 165.9835°W, 340 m depth; FAKU 121032, 1 (407.7 mm), 52.05°N, 179.9833°W, 125–1000 m depth; HUMZ 46218, 1 (264.6 mm), 51.8°N, 174.5167°W, 320 m depth, 16 June 1975; HUMZ 46224, 1 (352.2 mm), 51.8°N, 174.5167°W, 320 m depth, 16 June 1975; HUMZ 67372, 1 (220.3 mm), Bowers Bank, 53.95°N, 180°E; HUMZ 67373, 1 (199 mm), Bowers Bank, 53.95°N, 180°E; HUMZ 67655, 1 (136.5 mm), 51.45°N, 179.88°W; HUMZ 68524, 1 (343.6 mm), 52.1333°N, 171.6667°W, 490 m depth; FAKU 119256, 1 (316.8 mm), 54.6887°N, 166.675°W, 330 m depth; FAKU 119237, 1 (325.8 mm), 51.9333°N, 172.8167°W, 150–1030 m depth; FAKU 119239, 1 (245.1 mm), 51.9333°N, 172.8167°W, 150–1030 m depth; FAKU 119235, 1 (239.7 mm), 51.7667°N, 177.0333°E, 100–885 m depth; FAKU 119238, 1 (318 mm), 51.9333°N, 172.8167°W, 150–1030 m depth; FAKU 131034, 1 (283.8 mm), 53.7985°N, 166.3926°W, 249 m depth, 25 May 1996, FV *Golden Dawn*, J. W. Orr; HUMZ 88965, 1 (205.9 mm), 51.73°N, 177.86°E; HUMZ 88969, 1 (167.2 mm), 51.73°N, 177.86°E, 10 August 1980; HUMZ 89170, 1 (206.2 mm), 52.93°N, 169.33°W, 7 July 1980; UW 116497, 1 (300 mm), 52.499°N, 173.5789°W, 233 m depth, 2 June 2000, FV *Dominator*, J. W. Orr; UW 116488, 1 (265 mm), 52.2796°N, 170.6521°W, 160 m depth, 14 June 2006, FV *Gladiator*, J. W. Orr; UW 116550, 2 (195–200 mm), 51.8444°N, 173.9223°W, 267 m depth, 19 June 2006, FV *Gladiator*, J. W. Orr. **Gulf of Alaska:** UW 15167, 2 (173–241 mm), Alaska, Frederick Sound; UW 116883, 1 (365 mm), 56.279°N, 153.1482°W, 349 m depth, 23 March 2002, RV *Miller Freeman*, C. Wilson; UW 111744*, 9 (254–363 mm), 59.05°N, 143.0167°W, 300–600 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 116881*, 10 (265–410 mm), 59.05°N, 143.0167°W, 300–600 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 116882*, 9 (290–355 mm), 59.0333°N, 141.0167°W, 300–400 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 49325, 1 (372 mm), 59.0182°N, 141.1633°W, 313 m depth, 12 July 1999, FV *Morning Star*, J. W. Orr; UW 45237, 17 (292.5–411 mm), 59.0167°N, 147.033°W, 300–400 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 45235*, 6 (303–348.5 mm), 59.05°N, 143.017°W, 300–600 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 45236*, 8 (225.1–335 mm), 58.0667°N, 140.067°W, 300–400 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 113745, 1 (305 mm), 58.7708°N, 152.6504°W, 206 m depth, 6 July 2003, FV *Gladiator*, J.W. Orr; UW 114763, 1 (132.1 mm), 55.0236°N, 133.34°W, 189 m depth, 31 July 2005, FV *Sea Storm*; FAKU 121176, 1 (395.4 mm), 54.7833°N, 157.8167°W, 250 m depth; FAKU 121177, 1 (488 mm), 54.7833°N, 157.8167°W, 250 m depth; FAKU 121178, 1 (177.1 mm), 54.7833°N, 157.8167°W, 250 m depth; FAKU 121179, 1(489.4 mm), 54.7833°N, 157.8167°W, 250 m depth; FAKU 121180, 1 (217.7 mm), 54.7833°N, 157.8167°W, 250 m depth; HUMZ 102216, 1 (361.8 mm), 56°N, 149.25°W, 333 m depth, 15 August 1984; HUMZ 102577, 1 (337 mm), 56.55°N, 152.12°W, 152 m depth; HUMZ 102582, 1 (354.3 mm), 56.55°N, 152.12°W, 152 m depth; HUMZ

102583, 1 (351.5 mm), 56.55°N, 152.12°W, 152 m depth; UW 116493*, 1 (290 mm), 58.5212°N, 148.9618°W, 112 m depth, 31 July 2005, FV *Northwest Explorer*, J. W. Orr; UW 46069, 2 (335–353 mm), 54.9668°N, 157.2281°W, 308 m depth, 2 June 1999, FV *Morning Star*, J. W. Orr; UW 113672*, 4 (324.4–415 mm), 59.05°N, 143.0167°W, 300–600 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 111745*, 13 (196.6–445 mm), 59.05°N, 143.0167°W, 300–600 m depth, 2001, FV *Ocean Prowler*, D. Courtney; UW 115759, 3 (138.7–184 mm), 53.9637°N, 166.5257°W, 136 m depth, 6 June 2006, FV *Sea Storm*; UW 49336, 1 (246.7 mm), 53.2207°N, 169.7395°W, 328 m depth, 2 June 2002, J. W. Orr; UW 115763, 2 (131.2–140 mm), 57.9752°N, 137.1904°W, 282 m depth, 7 July 1999, J. W. Orr; UW 116885, 1 (430 mm), 58.3523 N, 147.828 W, 498 m gear depth, 1838 m bottom depth, D. E. Stevenson; UW 116884, 1 (455 mm), 58.3492 N, 147.825 W, 1089 m gear depth, 1892 m bottom depth, D. E. Stevenson. **Canada:** CAS 15392, 1 (155.3 mm), off Vancouver I., La Perouse Bank, 46°N, 126.5333°W, 421 m depth, 11 September 1972, RV *G.B. Reed*, T. Iwamoto. **Oregon:** CAS 40414, 3 (205.2–285 mm), 45.1717°N, 124.5117°W, 403 m depth, 5 September 1977, RV *Commando*; UW 43356, 1 (458 mm), 44.8898°N, 124.8867°W, 424 m depth, 19 October 1993, FV *Miller Freeman*, R. R. Lauth; UW 20964, 2 (376.4–385 mm), Brown Bear Seamount, 46.03°N, 130.4167°W, 480 m depth, RV *Pandora II*, D. W. Nelson; SIO 88-187, 1 (207.8 mm), 45.0607°N, 124.5152°W, 379 m depth, 8 December 1988, FV *Miller Freeman*, W. C. Flerx and R. Dotson. **California:** UW 114780*, 1 (430 mm), off Eureka, K.G. Thiesfeld; SIO 75-384, 1 (370 mm), Coronado Bank, 32.5817°N, 117.4°W, 11 April 1975, J. La Grange; SIO 75-386, 3 (385–420 mm), Coronado Bank, 32.581°N, 117.4°W, 421 m depth, 16 April 1975, J. La Grange; SIO 75-432, 1 (385 mm), Coronado Bank, 32.5833°N, 117.41°W, 11 February 1975, J. La Grange; SIO 75-433, 3 (385–405 mm), Coronado Bank, 32.5833°N, 117.41°W, J. La Grange; SIO 75-431, 1 (385 mm), Coronado Bank, 32.5833°N, 117.41°W, 7 February 1975, J. La Grange; SIO 73-434, 1 (377 mm), Coronado Bank, 32.5833°N, 117.41°W, 25 June 1975, J. La Grange; SIO 81-138, 1 (395 mm), 3 mi W of SIO Pier, in SIO Canyon, 32.85°N, 117.27°W, 457 m depth, 17 April 1975, Sullivan and McConnaughy. **Locality unknown:** FAKU-S 2848, 1 (341 mm); FAKU-S 2849, 1 (380.2 mm); FAKU-S 2989, 1 (279.7 mm); FAKU-S 2990, 1 (345 mm); FAKU-S 2991, 1 (333.2 mm); FAKU-S 3150, 1 (300 mm); FAKU-S 3166, 1 (343.1 mm); FAKU-S 3167, 1 (340.3 mm); FAKU 119231, 1 (416.6 mm); HUMZ 93754, 1 (408 mm).

Sebastes melanostomus USNM 77436, 1 (286.3 mm), syntype of *Sebastichthys intronigr*, California, *Albatross* Station 4410, "Long Point, Santa Catalina Island, N. 79 degrees W., 2.8 miles," 326–357 m depth, 11 April 1904; USNM 77437, 1 (300.2 mm), syntype of *Sebastichthys intronigr*, California, *Albatross* Station 4418, "off Santa Barbara I., SW. Rock Santa Barbara Island, N. 8 deg. E. 6.9 miles," 435–567 m depth, 12 April 1904.

Sebastes matsubarai USNM 48146, 1 (302 mm), Japan, Kushiri; FAKU 89180, 1 (392.3 mm), Japan; FAKU 89181, 1 (357.3 mm), Japan; FAKU 50100, 1 (291.1 mm), Japan; FAKU 89176, 1 (365.3 mm), Japan; FAKU 89177, 1(365.5 mm), Japan; FAKU 89178, 1 (398.6 mm), Japan; FAKU 89179, 1 (365.5 mm), Japan.

Sebastes baramenuke 3 FAKU 81605, 1 (409 mm), Japan; FAKU 50097, 1 (279.1 mm), Japan, Miyako Prefecture, 39.7°N, 142.5°E; FAKU 50098, 1 (288.3 mm), Japan, Miyako Prefecture, 39.7°N, 142.5°E.

Sebastes iracundus FAKU 81604, 1 (446.8 mm), Japan.

Sebastes flammeus FAKU 81606, 1 (364.9 mm), Japan; FAKU 81607, 1 (385.6 mm), Japan; FAKU 88906, 1 (555.4 mm), Japan.

The influence of elemental chemistry on the widths of otolith increments in the neon damselfish (*Pomacentrus coelestis*)

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Abstract—We examined the potential for water chemistry to affect the width of daily increments in reef fish otoliths using both mensurative and manipulative methods. We found significant differences in the widths of increments in otoliths of the neon damselfish (*Pomacentrus coelestis*) collected in different habitats at One Tree Island on the Great Barrier Reef. We then used manipulative experiments to determine if natural water masses (ocean water vs. lagoon plume) could produce different incremental widths in otoliths in the absence of potentially confounding factors. Fish exposed to ocean water had significantly wider otolith increments for two of the three experiments. Elemental analyses indicated that Ba/Ca ratios were significantly correlated with increment widths for two of the three experiments and Sr/Ca ratios did not correlate with increment width for any experimental period. Variation in crystal-lattice orientation did not explain differences in increment width between treatments. Differences in water chemistry can affect increment widths in otoliths of reef fishes, potentially confounding patterns previously attributed to growth rate or condition alone.

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Recent research has elucidated the importance of presettlement condition and growth rate of reef fish for survival and recruitment (Bergenius et al., 2002; Hoey and McCormick, 2004; Sponaugle and Pinkard, 2004). Studies on the condition of presettlement fishes have been based on a number of measures that include growth rate, biochemical development, and behavior (McCormick, 1998). More commonly, however, widths of increments in otoliths have been used as a means of examining growth on the assumption of a good relationship between somatic and otolith growth (Campana, 1999; Meekan et al., 2003). In addition, otolith increment widths may provide information about the condition of presettlement fishes; it has been demonstrated that fish with small lipid reserves generally deposit narrower increments than fish with adequate lipid reserves (Paperno et al., 1997; Molony and Sheaves, 1998). Indeed, periods of starvation are known to have a substantial influence on the increment widths of otoliths (Moksness, 1992; Molony, 1996), as can water temperature (Hüssy et al., 2003; Powell et al., 2004) and the onset of metamorphosis into a juvenile form (Wilson and McCormick, 1999). Thus, the increment widths of otoliths are an important, but not necessarily simple tool, by which larval condition and growth can be assessed.

Otoliths are composed of an aragonite lattice deposited on a protein matrix; this lattice incorporates trace elements, derived mainly from the surrounding water mass (Walther and Thorrold, 2006), as deposition occurs (Campana, 1999). Indeed, there is a close link between water and otolith chemistry for some elements (i.e., Ba; Bath et al., 2000; Walther and Thorrold, 2006); this relationship allows otolith chemistry to be used as a proxy for water chemistry. Although the process of elemental deposition is not entirely understood at this time, it is possible that the chemical composition of the water mass may influence increment widths in the otoliths by means of otolith chemistry and related crystallography, thus potentially confounding the interpretation of these increments.

Furthermore, some reef fish are capable of distinguishing and actively choosing particular water masses (Doherty et al., 1996; Atema et al., 2002; Gerlach et al., 2007), presumably as a means of choosing suitable habitat at settlement. This study demonstrates that otolith increment widths can vary between fish in dif-

ferent water masses that have a constant temperature and offer constant food conditions. Water masses, therefore, have the potential to influence increment width by affecting growth rates (Sponaugle and Pinkard, 2004) or influencing otolith chemistry; the challenge is to distinguish between these two mechanisms.

Materials and methods

This study was conducted on One Tree Island (OTI) in the southern Great Barrier Reef (GBR), Australia. This platform reef is closed off from the surrounding ocean for 3–5 hours during each low tide (Ludington, 1979). Thus, the geochemical properties of the lagoon water may be distinct from the surrounding ocean water (Atema et al., 2002); this feature may influence the increment widths of the otoliths of the fish residing in the lagoon. The otoliths of newly settled neon damselfish (*Pomacentrus coelestis*) collected from different water masses around OTI (i.e., reef slope (=oceanic water) vs. lagoon water) were examined and the increment widths were measured. A series of manipulative experiments were conducted with oceanic and lagoon water to examine the effects on increment widths of *P. coelestis* otoliths in the absence of potentially confounding factors, including food and temperature. The increment widths derived from these experiments were then compared with the results of another study (Patterson et al., 2004a) where otolith chemistry of the same experimental fish was examined as that used in this study to determine if otolith chemistry may affect increment width. In addition, scanning electron microscopy (SEM) was used to examine otolith microstructure because variation in elemental chemistry may potentially alter crystal orientation.

Wild fish

Newly settled *P. coelestis* were collected by divers using hand nets and clove oil from two sites on the reef slope (ocean 1 and 2) and two sites within the lagoon (lagoon 1 and 2) at OTI (23°30′S, 152°06′E; n=7 per site). The sagittae were removed and one was randomly chosen for analysis. Transverse sections were prepared by embedding the sagittae in Epofix resin (Struers, Milton, Australia) and polishing with 800-grit-size lapping paper on a grinding wheel and then fine-polishing with 3-μm lapping film until the primordium was visible and daily rings could be distinguished. The daily increments (validated by Flood, 2000) were counted and measured with a Leica image analysis system (Leica Microsystems, North Ryde, NSW, Australia). A drop of immersion oil was placed on each otolith to enhance its appearance, and an image of each otolith was captured and saved at a magnification of 400× with a Leica DC300 digital video camera. The distance between each increment on the distal side of each otolith was measured with the use of the software package Leica IM50.

Experimental fish

Experimental procedures were identical to those described in Patterson et al. (2004a), but are briefly repeated here. Presettlement (*sensu* Kingsford et al., 2002) *P. coelestis* were collected by using two to four moored light traps at several locations within 100 m of the reef slope (see Patterson et al., 2004a, Fig. 1) during three consecutive austral summer field seasons (January and February, 2000–02). Light traps were illuminated for three hours each night (21:00–22:00, 00:00–01:00, and 03:00–04:00) and were checked on the first high tide after sunrise. Samples from the light traps were sorted on the boat, and target species were taken to the laboratory to be counted and identified. Fish used in each experiment were collected over a period of two days. Additional individuals were immediately frozen to use as control fish (see *Crystallography* section).

The experimental design for each year differed only in the number of replicate fish placed in each tank at the beginning of the experiment (2000, n=6; 2001, n=10; 2002, n = 3; n varied because of among-year differences in the availability of fish). Experimental fish were allowed a minimum of 24 hours to acclimate to experimental conditions before the start of the experiment. It was not possible to measure the standard length (SL) of fish before the start of the experiment because this process stressed, and in some cases damaged the fish, which could have confounded the results. All fish were approximately the same size at the beginning of the experiment (~15 mm SL). Three replicates of each treatment (lagoon water vs. ocean water) were set up in 200-L tanks and fish were randomly placed in each tank. Aerated tanks were kept under shade cloth in a wet laboratory and were randomly positioned. Water (from lagoon ebb tide plumes and oceanic waters collected outside of plumes; see Patterson et al., 2004a, Fig. 1) was changed daily (~90%) and debris and potential food items were removed with a 30-μm net. The water had adequate time (~1 h minimum) to come to a standard experimental temperature before being placed in the tanks. Natural temperature variations were therefore controlled for. Fish were fed twice a day (am and pm) with cultured *Artemia* sp. and wild zooplankton collected with channel nets or with a 120-μm net during horizontal tows. Quantities of food were measured (500 mL per feeding time) and fish were fed in random order at each feeding time to eliminate any potential bias in food allocation. The water temperature was recorded at each feeding session. No differences were detected in water temperature during the experimental treatment (mean conditions for all years and treatments ±standard error [SE]: am feeding: 26.5°C ±0.09, pm feeding: 28.2°C ±0.09). For all years of the experiment, there were no significant differences in water temperature by tank nested in treatment (ANOVA: am: $F_{1,4}$=0.09, P>0.05; pm: $F_{1,4}$=0.06, P>0.05) or by treatment (ANOVA: am: $F_{1,4}$=2.61, P>0.05; pm: $F_{1,4}$=5.13, P>0.05). Fish were maintained for a total of nine days in each experiment (i.e., nine days per year) after which three fish

from each tank were randomly selected and frozen (−20°C) for microstructural analysis of otoliths.

The SL of the *P. coelestis* used in the experiment (mean=15.2 mm ±0.07 SE) did not differ between treatment groups for any year of the experiment (ANOVA: 2000: $F_{1, 16}$=3.31, P>0.05; 2001: $F_{1, 16}$=1.43, P>0.05; 2002: $F_{1, 16}$=2.4, P>0.05) or among years (ANOVA: $F_{2, 51}$=0.24, P>0.05). The otoliths (the left or right sagittae was chosen randomly), were rinsed three times in Milli-Q water (Millipore, Billerica, MA). and were allowed to dry. Otoliths were polished as described above (see *Wild fish* section. Following elemental analysis of the otoliths by means of laser ablation inductively coupled mass spectrometry (for a complete description of the elemental analysis see Patterson et al., 2004a), the daily increments were counted and measured in the same manner as described earlier. Note that all increments were counted and measured, not just those pertaining to the experiment. Settlement marks, based on the criteria defined by Wilson and McCormick (1999), were also noted.

Crystallography

The orientation of crystals was examined with SEM near the sulcus of sectioned sagittae. The surface of sectioned otoliths was lightly etched (EDTA 0.1 to 0.01M for 3 minutes) to observe the orientation of the crystal-lattice. Although etching altered the edge of crystals, orientation of the long-axis of the crystal-lattice was still easily seen (e.g., Linkowski et al., 1993). The crystal-lattice was observed for four fish from each treatment (ocean vs. lagoon) for the year 2000 experiment. The crystal-lattice of experimental fish was compared with that of 18 control fish. These fish were collected in the same light traps as fish used in the experiments, but were not subjected to any experimental conditions.

Statistical analysis

Data were tested for homogeneity of variances with a Cochran's C-test and were found to be homogeneous. Wild fish otolith increments were analyzed with a nested ANOVA design with the factors habitat (ocean vs. lagoon) and site nested within habitat. However, because site nested within habitat was found to be nonsignificant at the P = 0.25 level, sites were pooled with the residual to increase degrees of freedom and the ANOVA was undertaken again with only habitat as a factor (Underwood, 1997).

To analyze otolith increments corresponding to both the pre-experimental (nine days before the start of the experiment) and the experimental phase (nine days of the experiment), fully nested ANOVAs were used for each year of the experiment. This design used two factors (treatment and tank nested within treatment). Treatment was a fixed factor and tank was a random factor. One-factor ANOVAs and Tukey's HSD *post hoc* tests were used to examine differences among years within treatment groups. Paired t-tests were also used to examine the relationships of increment widths before and during the experiment for each experimental treatment. Based on the findings of Patterson et al. (2004a), correlation analyses were used to examine the relationships between incremental widths and elemental ratios (i.e., Ba/Ca, Sr/Ca).

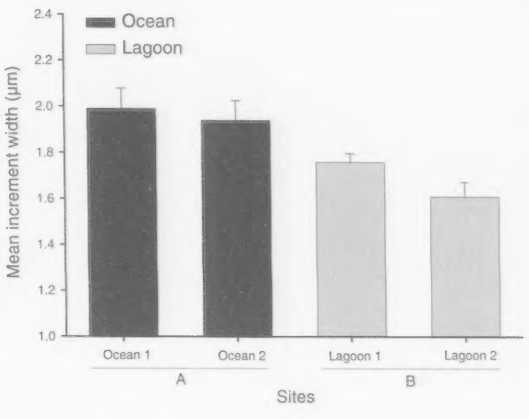

Figure 1

Mean (+1 standard error) increment width (µm) in otoliths of nonexperimental neon damselfish (*Pomacentrus coelestis*) (n=7 per site) collected from two sites on the reef slope in ocean water (Ocean 1 and 2) and within the lagoon (Lagoon 1 and 2) at One Tree Island, Great Barrier Reef, Australia. Tukey's HSD contrast groupings are indicated as letters (i.e., A, B) where overall differences were significant (P<0.05).

Results

Neon damselfish in natural habitats displayed significant variation in increment widths; increments were narrower in otoliths of fish collected in the lagoon (ANOVA: $F_{1, 26}$=14.76, P<0.001; Fig. 1). For experimental fish, which all originated in ocean water, no difference in incremental spacing was found before the start of the experiment by treatment or by tank nested within treatment for any year of the experiment (ANOVA: 2000: $F_{1, 4}$=1.34, P>0.05; 2001: $F_{1, 4}$=0.17, P>0.05; 2002: $F_{1, 4}$= 0.04, P>0.05; Fig. 2). In addition, no difference in Ba/Ca ratios was found before the experiment (Fig. 3; Patterson et al., 2004a). Fish in ocean water in 2000 and 2002 had wider increments than those in the lagoon treatment groups (ANOVA: 2000: $F_{1, 4}$=28.53, P <0.01; 2001: $F_{1, 4}$= 1.62, P>0.05; 2002: $F_{1, 4}$=26.42, P<0.01), as well as a significant difference in Ba/Ca ratios (Figs. 2 and 3; Patterson et al., 2004a). In addition, there was a significant difference in increment widths among tanks within treatments for 2002. A Tukey's HSD test (P<0.05) indicated this was due to a tank of lagoon water where fish

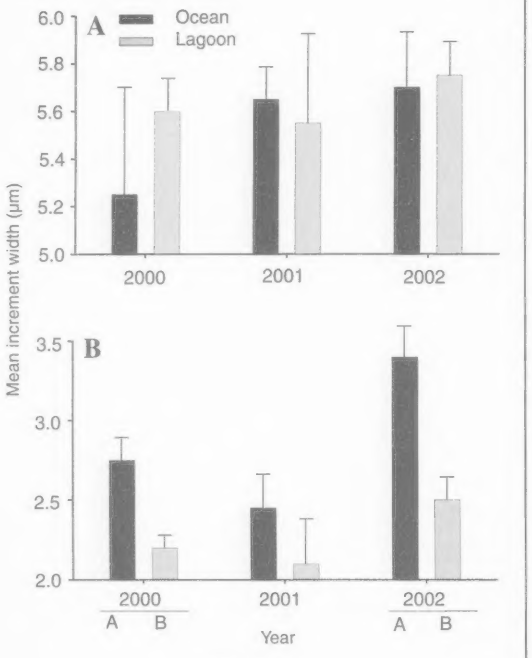

Figure 2

Mean (+1 standard error) increment width (μm) in otoliths of neon damselfish (*Pomacentrus coelestis*) (n=9 per treatment per year) for all years from ocean and lagoon treatments (**A**) before the start of the experiment and (**B**) during the experiment for each year of the experiment. Tukey's HSD contrast groupings are indicated as letters (i.e., A, B) where overall differences were significant (P<0.05). Note the difference of the y-axis scale in A and B.

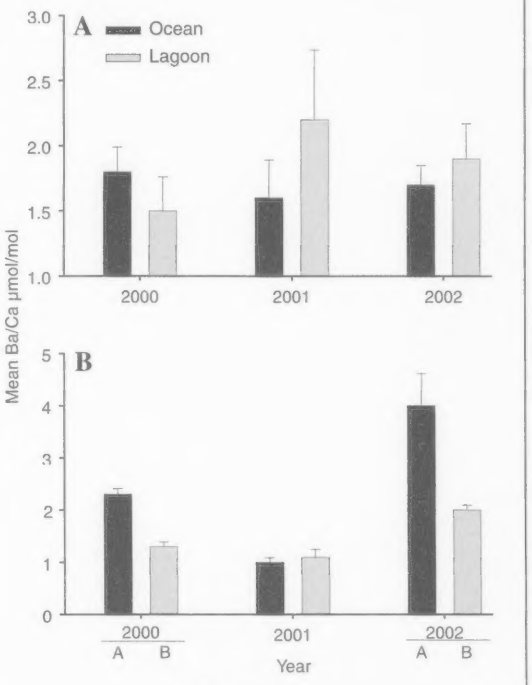

Figure 3

Ba/Ca ratios from otoliths of neon damselfish (*Pomacentrus coelestis)* (n=9 per treatment per year) for all years from ocean and lagoon treatments (**A**) before the start of the experiment and (**B**) during the experiment. Tukey's HSD contrast groupings are indicated as letters (i.e., A, B) where overall differences were significant (P<0.05). Note the difference of the y-axis scale in A and B.

had a higher mean increment width than the fish in the other two tanks (2.8 μm vs. 2.4 μm and 2.4 μm), but this did not obscure major differences between treatments. No significant difference in increment widths between treatment groups was detected for 2001. Finally, a significant difference in increment widths was detected among years for the ocean treatment group, but not for the lagoon treatment group (ANOVA: ocean: $F_{2, 24}$=16.56, P<0.05; lagoon: $F_{2, 24}$=2.28, P>0.05). Fish in the 2002 ocean treatment group had the widest increments of any fish in the experiments. Individual paired t-tests indicated that for both ocean and lagoon treatment groups there was a significant difference in increment width between the pre-experimental and the experimental increments (paired t-test: ocean: T_{242}=90.45, P <0.05; lagoon: T_{242}=322.05, P<0.05).

There were significant relationships between mean increment widths and Ba/Ca ratios for two of the three experimental years (2000: Pearson correlation coefficient, r=0.644, P<0.01; 2001: r=0.052, P>0.05; 2002: r=0.671, P<0.01; Fig. 4). There was no significant correlation between increment widths and Sr/Ca ratios for any of the experimental years (2000: Pearson coefficient, r=–0.417, P>0.05; 2001: r=0.033, P> 0.05; 2002: r=0.266, P>0.05; Fig. 4).

SEM indicated that the aragonite crystal-lattice within experimental otoliths was orientated perpendicularly to daily increments (Fig. 5). This pattern was consistent for ocean and lagoon treatments (angle from daily increments: ocean: 90° ±0°; lagoon: 90° ±0°) and control fish (90° ±0°). Variation in increment width could only be explained by variation in the length of the intra-incremental lattice rather than by a change in orientation or crystal packing.

Discussion

The pattern of wider increments observed for experimental *P. coelestis* held in ocean waters was consistent with

the pattern observed for wild fish. Additionally, these experiments demonstrated that increment widths could be altered in the absence of food and temperature differences. There are a number of factors that can potentially influence the increment widths of otoliths, such as feeding regime (McCormick and Molony, 1992), lipid reserves (Paperno et al., 1997; Molony and Sheaves, 1998), the onset of metamorphosis (Wilson and McCormick, 1999), water temperature (Powell et al., 2004), and metabolic rate (Mosegaard et al., 1988; Wright et al., 2001). Temperature and food, the most well-studied factors, were not relevant here because both were held constant during the experiments. Additionally, metabolic rates were expected to be consistent among individuals, given the similar size, age, and the constant temperature and feeding regimes of these individuals. It is also unlikely that metamorphosis played a role; experimental fish immediately "settled" in the tanks. Settlement marks were noted to form with narrower post-settlement increments, consistent with what is known for this species (Wilson and McCormick, 1999). However, these narrower increments were unlikely to have affected the results as they occurred in all fish in the experimental treatments over all years.

It is possible that the differences noted in increment widths were related to initial size or the condition of the fish during the presettlement phase. Although the standard length of the fish was measured after the study and no differences were found, it was not possible to measure fish before the study (see Methods) and more explicit measures of condition (e.g., Fulton's *K*; Hoey and McCormick, 2004) were not applied before the experiment. Therefore, pre-existing differences in size or condition may have influenced the results to some degree (e.g., some fish may have started the experiment with higher lipid reserves which could have influenced their growth rate and increment widths irrespective of experimental conditions). The randomized allocation of fish between treatments on three separate occasions, however, makes this scenario highly unlikely. In addition, any size or conditional differences were more likely to have introduced variation within the treatment groups rather than the between treatment differences observed in this study.

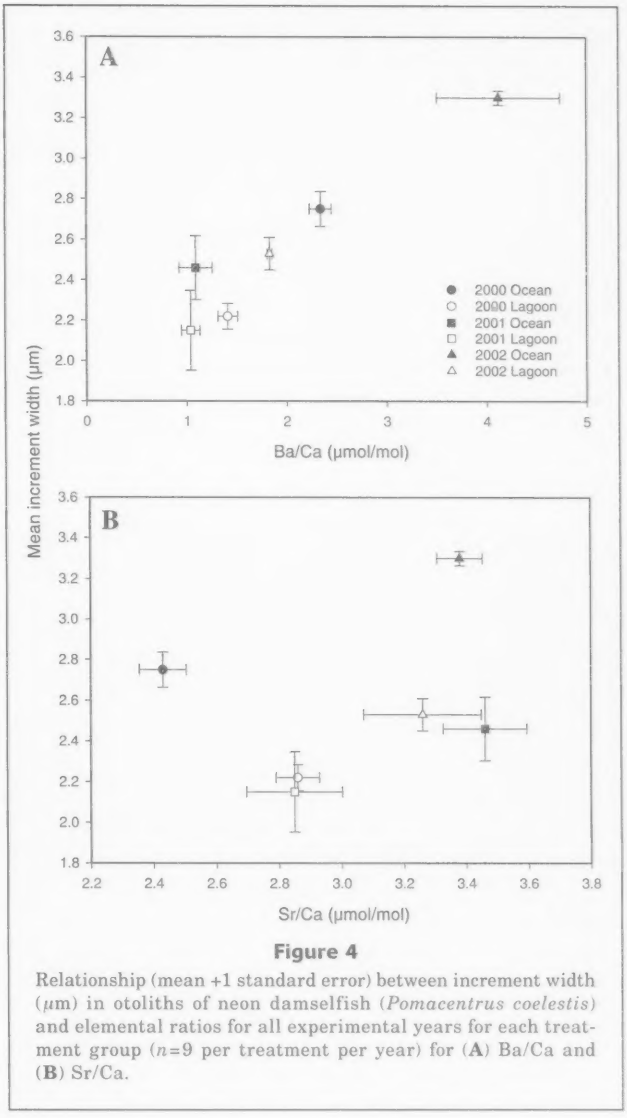

Figure 4

Relationship (mean +1 standard error) between increment width (µm) in otoliths of neon damselfish (*Pomacentrus coelestis*) and elemental ratios for all experimental years for each treatment group (n=9 per treatment per year) for (**A**) Ba/Ca and (**B**) Sr/Ca.

Water chemistry may have influenced increment width in an indirect manner. Late-stage reef fish have well-developed olfactory systems and are capable of detecting chemical differences of ocean and lagoon waters found around OTI (Atema et al., 2002). Some taxa prefer lagoon water (e.g., apogonids), whereas it has been shown that *P. coelestis* actively avoid settling in the lagoon and prefer the coral rubble habitat found on reef slopes (Doherty et al., 1996). Indeed, *P. coelestis* were relatively rare in the lagoon at OTI, and this finding confirms the previous report of Doherty et al. (1996). Although not well-studied in reef fish (but see Arvedlund et al., 1999 and Gerlach et al., 2007), natal imprinting and homing has been documented in fish species, most notably in the olfactory-driven homing of salmonids (Dittman et al., 1996). Because *P. coelestis* are likely spawned and undergo their presettlement life outside of lagoon habitats, they may have imprinted on nonlagoon waters. Lagoon water, therefore, may be an indication of substandard habitat that *P. coelestis* actively avoid. Holding fish in lagoon water may have triggered a physiological response (e.g., stress), resulting in reduced growth and narrower otolith increment widths for fish held in nonlagoon waters (e.g., Marchand et al., 2003). However, this seems unlikely to have been

the primary mechanism driving our results. Although it was not possible to measure fish before each experiment, there was no difference in standard length between treatment groups for any year, indicating that fish in both groups had similar growth rates.

Alternatively, it is possible that lagoon water lacks some physiologically important trace element which limits growth. Again, this seems unlikely to fully account for our results because *P. coelestis* do inhabit and spawn in the lagoon, albeit not in great numbers, as do other pomacentrid species. This would not be expected if lagoonal waters were lacking an element of such physiological importance. Furthermore, Ba is not this limiting element because it has been clearly shown that Ba concentrations in otoliths are indicative of Ba concentrations in water (Bath et al., 2000; Walther and Thorrold, 2006) and that Ba is not influenced by the physiology of the fish (Campana, 1999). Because there was a significant relationship between increment widths and Ba/Ca, it seems likely that another mechanism was involved in producing the results observed.

A related study, in which the otolith chemistry of the same otoliths used here was examined, indicated significant differences in the otolith chemistry of the two treatment groups for 2000 and 2002, primarily driven by differences in Ba/Ca and Sr/Ca (Patterson et al., 2004a). Similar to the results of the present study, no difference in otolith chemistry between the treatment groups was detected for 2001. The differences in otolith chemistry between the treatment groups probably reflected differences in the composition of the water masses (Bath et al., 2000). Oceanographic features that may affect water chemistry (i.e., upwelling, phytoplankton blooms; Patterson et al., 2004a, 2004b) are not temporally stable. This may explain why significant differences in otolith chemistry and microstructure were found only for fish from the 2000 and 2002 experiments. In addition, there was a significant relationship between Ba/Ca ratios and increment widths, and annual variation in increment widths reflected differences for Ba. No significant correlation was found between Sr/Ca ratios and incremental widths, which concurs with previous studies (Kalish, 1989; Gallahar and Kingsford, 1992).

The mechanisms by which trace elements are deposited into otoliths are not entirely understood at this time. Therefore, the exact mechanisms by which otolith chemistry may directly influence increment widths remain unknown. Otoliths are composed primarily of calcium carbonate in the form of aragonite. Divalent metal ions of similar size to Ca, such as those of Ba and Sr, can substitute directly for Ca in the crystal-lattice (Campana, 1999). However, other elements such as Zn and Mn may occupy different positions in the otolith matrix (i.e., in the interstitial spaces or in association with the proteinaceous matrix; Campana, 1999). Although it is known that otolith crystal orientation is protein-mediated (Lowenstam, 1981), it is not known how physiological stress can affect protein deposition, or how trace element inclusion may influence increment width. The orientation of the crystal-lattice did not change in fish treated with ocean and lagoon waters and crystal orientation was similar to other fishes (e.g., Linkowski et al., 1993). Elemental availability in the location of the trace metal inclusions may be capable of creating differences in increment width by increasing the length, or number, of

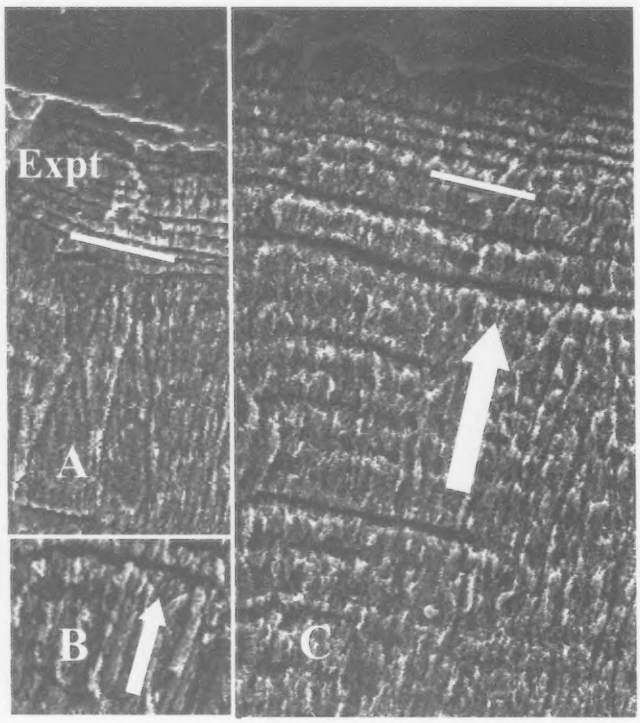

Figure 5

Scanning electron microscope images of the crystal lattice in otoliths of neon damselfish (*Pomacentrus coelestis*). (**A**) Image of an otolith of an experimental (Expt) fish. "Expt" indicates the area (above the white line) where increments were laid down in the otolith of a fish exposed to ocean waters. (**B**) Detail of crystal-lattice from a control fish; (**C**) Detail of the crytalline lattice of a postsettlement control fish; the white line indicates the settlement mark. Large white arrows indicate the orientation of the crystal-lattice. Scale: increments near the edge of the otolith are separated by approximately 1.5 μm.

crystals that are perpendicular to incremental zones, or by altering the way in which crystals are bundled (tightly packed vs. chaotic bundles). The SEM used in this study did not have the resolution to distinguish between these alternatives.

In summary, this study has provided convincing evidence that water chemistry can affect the increment widths of reef fish otoliths by either direct or indirect mechanisms. It is also possible that multiple mechanisms have interacted to produce the results observed. Although the results presented here are preliminary, they indicate that interpreting increment widths may be more complex than has previously been noted and, at least under some circumstances, differences in water chemistry may confound patterns previously attributed only to growth and condition. Further research on this topic is therefore warranted and should include controlled experiments with temporally comparable measurements of water chemistry, otolith chemistry, and increment widths to tease apart the complex mechanisms at work. Lagoons are also found on other reefs (Atema et al., 2002), and further studies using other water masses are needed to determine if the results presented here are prevalent in coral reef fish or are specific to the system at One Tree Island. Finally, continuing research on natal imprinting may elucidate to what extent reef fish imprint on specific water masses, and the physiological consequences of that imprinting.

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Seasonal, diel, and lunar spawning periodicities and associated sound production of white seabass (*Atractoscion nobilis*)

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Abstract—Spawning periodicities of white seabass (*Atractoscion nobilis*) were evaluated by observing spawning behavior, by collecting eggs, and monitoring recognizable sounds produced during the release of gametes. A total of 297 spawning events were documented from 15 male and 47 female white seabass contained within the seminatural confines of a 526-m^3 net pen located in Catalina Harbor, Santa Catalina Island, California. Consistent spawning occurred from March through July 2001–03, and peaked in May at a photoperiod of 14 hours. Most spawning occurred within the 2-hour period following sunset or from 19:00–20:00 hours Pacific Standard Time. White seabass spawned at every phase of the lunar cycle; but an increase in successive spawning events followed the new moon. Most spawning occurred in water temperatures from 15 to 18°C, and there was no apparent correlation with tidal cycles. Seasonal and diel spawning periods were directly correlated with increases in the rate, intensity, and variety of white seabass sounds; this correlation may indicate that sounds function to enhance reproductive success. These findings can be extended to further develop seasonal fishery regulations and to better comprehend the role of sound in the reproduction of sound-producing fishes.

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The white seabass (*Atractoscion nobilis*), also known as white weakfish (Eschmeyer et al., 1983), is an oviparous group spawner; multiple males fertilize the eggs of a gravid female as gametes are broadcast into the water column (Aalbers and Drawbridge, in press). As the largest member of the family Sciaenidae (croakers and drums) that inhabits California coastal waters (Miller and Lea, 1972), white seabass produce relatively large eggs, averaging 1.27 mm in diameter (Moser et al., 1983). Fertilized eggs are buoyant and drift with the ocean surface currents for two days before hatching into planktonic larvae that disperse for approximately 30 days and settle out nearshore at a size of 7–10 mm (Moser et al., 1983; Allen and Franklin, 1992).

A previous study, in which the gonads of commercially caught white seabass were examined, indicated that spawning occurs from April through August and peaks in May and June (Skogsberg, 1925). The majority of white seabass have traditionally been landed in the late spring and early summer, when spawning aggregations develop nearshore and around coastal islands (Skogsberg, 1939; Thomas, 1968). Following a sharp decline in California landings, the commercial harvest of white seabass was restricted from March 15 through June 15 to protect spawning stocks, while recreational bag limits were reduced from three to one fish during this three-month period. Although current restrictions offer white seabass spawning aggregations some refuge, a better understanding of spawning periods will allow managers to further develop seasonal fishery regulations.

Reproduction has been coupled with sound production in other species of Sciaenidae, including the red drum (*Sciaenops ocellata*) (Guest and Lasswell, 1978), spotted seatrout (*Cynoscion nebulosis*) (Mok and Gilmore, 1983), weakfish (*Cynoscion regalis*) (Connaughton and Taylor, 1995), and orangemouth corvina (*Cynoscion xanthulus*) (Fish and Cummings, 1972). Typical of most sciaenids, male white seabass possess sonic musculature that resonates pulsed sounds from the adjacent gas bladder (Tavolga, 1964). Although the sonic musculature and gas bladder structures vary considerably among the 270 sciaenid species (Chao, 1986; Ramcharitar et al., 2006), the sonic structures that Smith (1905) and Tower (1908) described a century ago in male weakfish closely resemble those of white seabass. The importance of sound to this diverse family has been well documented during periods of reproductive activity (Ramcharitar et al., 2006); however, recognizable sounds during the actual release of gametes

have only been described in the white seabass (Aalbers and Drawbridge, in press).

Acoustically monitoring the sounds produced by spawning fish can reveal the periodicity and critical habitat of spawning aggregations and help managers in the development of fishery regulations and in the better placement of marine protected areas (Luczkovich et al., 1999). However, patterns of spawning-related sound production must be established in order to yield meaningful results for such application. The objective of this investigation was to determine the spawning periodicities and associated sound production patterns of white seabass under seminatural environmental conditions. This study presents a comprehensive assessment of sciaenid sound production as it relates to known spawning activity and establishes the foundation from which passive acoustic surveys can be conducted.

Materials and methods

Sampling locations and procedures

Research was conducted from an aquaculture platform consisting of four 9×9×6.5-m net pens moored in Catalina Harbor, Santa Catalina Island, CA. Forty-one adult white seabass were transported live to an individual net pen (526 m^3) from March through June 2000. An additional 21 fish, collected in May 2001, were excluded from the 2001 spawning data while being acclimated in a separate net pen. In October 2001, all fish were measured to the nearest mm, weighed to the nearest 10 g, examined to determine sex, tagged with a passive integrated transponder (PIT; Avid, Greeley, CO), and combined into a single net pen. Forty-seven female and 15 male fish ranged in initial size from 83 to 126 cm total length (TL) and 4.9 to 19.5 kg in weight. Fish were only manipulated when remeasured in February 2003 and no mortality occurred during the study. Captive white seabass were fed variable rations of freshly caught squid (*Loligo opalescens*), chub mackerel (*Scomber japonicus*), and jack mackerel (*Trachurus symmetricus*) as they became available locally.

Documentation of spawning

Fish spawning periodicity was assessed from 2001 to 2003 by direct observation from an elevated deck, by sampling the net pen for freshly spawned eggs with a 500-μm-mesh dip net, and by acoustically monitoring fish sounds. Successive spawning events were difficult to differentiate from egg sampling alone; therefore multiple spawning events were documented only if visually observed or acoustically detected and accompanied by increased egg abundance in dip-net samples in comparison to previous samples. Gravid females and courting males were occasionally scanned for individual identification with a PIT (passive intergrated transponder) reader when fish approached the surface.

Analyses of spawning data

Date and time of day, photoperiod, lunar phase, and tidal cycle were noted upon detection of a spawning event. Data were grouped by month, hour, and phase of the synodic month to determine seasonal, diel, and lunar spawning periodicities. Hourly spawning periodicity was analyzed in relation to sunset and to the 24-h clock after correcting to Pacific Standard Time (PST), without adjusting for daylight savings time. Water temperature was recorded to the nearest 0.15°C every hour at a depth of 3 m with an Optic Stowaway temperature logger (Onset Computer Corp., Pocasset, MA) suspended from the net pen. Continuous temperature data for the 2002 spawning season were obtained from a temperature logger at an adjacent net-pen site (300 m away) within Catalina Harbor. Additional temperature and salinity measurements were made upon detection of eggs with a YSI 85 instrument (YSI Inc., Yellow Springs, OH).

Acoustic equipment

Sounds were received with an omnidirectional hydrophone (International Transducer Corporation 6050-C, Santa Barbara, CA) with an essentially flat frequency response between 50 Hz and 35 kHz. The hydrophone was powered by a custom 24-V power supply and amplifier with an incorporated reference tone (Frank Aubrey, Hubbs-SeaWorld Research Institute, San Diego, CA). Sounds were recorded with a PCM-M1 digital audio tape recorder (Sony Corp., Tokyo, Japan) set to a constant gain.

Audio recordings

Three-minute audio recordings were made at regular intervals with the hydrophone suspended at a depth of 3 m in the center of the net pen. Evening sound recordings, taken on 17 sampling dates from March through July 2003, were focused around periods of spawning activity, and individual recordings were made every 30 minutes from 1 hour before to 3 hours after sunset. Additional recordings were made every hour for 24 hours, once a month, on randomly selected dates from April 2003 through March 2004 to document seasonal and diel sound patterns. No recordings occurred in February 2004 because of extreme weather conditions.

Acoustic analyses

Sound recordings (n=404) were digitally transferred to a PC hard drive for spectral analysis (SpectraPlus, vers. 2.32.04, Pioneer Hill, Campbell, CA). Based on spectral characteristics, the following categories of white seabass sounds were noted: single pulse-trains, multiple pulse-trains, drum-rolls, thuds, abbreviated chants, spawning chants, or hydrodynamic booms as described in Aalbers and Drawbridge (in press). Sound production rates for each sound type were determined from each recording.

The most intense sounds, as recorded, of each type were analyzed against a 1-kHz reference tone on each 3-min recording. Sound intensities were scaled with values consistent to one another, but not as absolute sound pressure level (dB re 1μPa) because the entire hydrophone system had not been calibrated recently. The terms "level" and "intensity" are used synonymously to convey the relative data presented here.

Statistical analyses

A one-way analysis of variance (ANOVA) was used to examine whether diel spawning times were consistent throughout the spawning season both in relation to sunset and the 24-h clock. The numbers of spawning events observed at each phase of the lunar cycle were analyzed against expected occurrences by using a chi-square test. Sound rates from 24-h recordings during the spawning season (March–July) were compared to those when there was no spawning (August–January) using a two-sample *t*-test. Two-sample *t*-tests were also used to compare the rates and relative levels of sounds from evening recordings made on days that spawning did and did not occur.

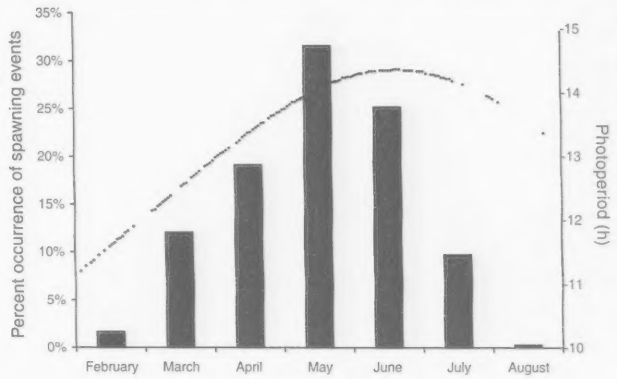

Figure 1

Monthly frequency distribution and photoperiods (◆) of spawning events for 297 white seabass (*Atractoscion nobilis*) spawning events from 2001 through 2003.

Results

Seasonal spawning

Over the three-year study, 297 spawning events were documented; 53, 146, and 98 events were recognized in 2001, 2002, and 2003, respectively. The longest spawning season extended between 22 February and 15 August 2002, whereas the other spawning seasons ranged from 17 March to 27 June 2001 and from 3 March until 16 July 2003. Of the documented spawning events, 76% occurred from April through June, and 98% from March through July over the three-year period. Spawning peaked in May as photoperiod increased to 14 hours (Fig. 1).

Diel spawning

Spawning occurred from two hours before sunset until 4.5 hours after sunset. Sixty-five percent of spawning activity occurred over the two-hour period following sunset and 91% occurred within the four-hour period spanning from one hour before sunset to 3 hours after sunset (Fig. 2A). A significant shift in the diel spawning periodicity in relation to sunset as the spawning season progressed occurred in all three years (ANOVA, F=8.78, df=4, P>0.001). For instance, in March 2002 the average spawning time was between two and three hours after sunset; however, by July 2002 the average spawning event occurred within the first hour after sunset. The inverse seasonal trend was observed when spawning times were analyzed in relation to sunrise. No equivalent seasonal shift in the time of spawning occurred when events were analyzed in relation to the 24-hour clock (ANOVA, F=2.41, df=4, P=0.06). Spawning peaked between 19:00 and 20:00 h PST in all three seasons and was not confirmed before 17:30 or after 22:00 h (Fig. 2B).

Lunar spawning

Spawning occurred at every phase of the lunar cycle, but not uniformly (Fig. 3, χ^2=49.57, df=29, P=0.01). The greatest amount of spawning occurred from the new moon until four days after the new moon; 63% of documented mass spawning events (dates with at least four successive spawning events) took place during this five-day period.

Successive spawning events were common and as many as eight events occurred in a single night, presumably from different females. The time between successive spawning on 21 sampling dates ranged from 1 to 98 minutes (mean=31 min, n=66). A female individual was identified with the PIT reader before spawning on 12 June 2002 and again on 16 June 2002.

Environmental factors

Ambient water temperatures ranged seasonally from 11.3° to 21.7°C over the study period. Spawning occurred between 12.5° and 20.8°C, and bimodal peaks in the percent occurrence of spawning were apparent at 15° and 18°C. Seventy-six percent of spawning occurred in water temperatures from 15° to 18°C. Salinity at the study site ranged from 34.2° to 34.8 ppt. Spawning occurred throughout the range of tidal cycles with no apparent tendency towards ebb or flow. The ratio of

spawning that occurred around high tide was equivalent to the ratio of high tides that occurred within the fundamental diel spawning period (1 hour before to 3 hours after sunset).

Sound production

The sound production rate of white seabass peaked in May, averaged 29.2 sounds/min, and reached a minimum average of 1.5 sounds/min (primarily hydrodynamic booms) in December (Fig. 4). Sound production rates were significantly greater on 24-h recordings made during the spawning season (mean=12.7 sounds/min) than during months without spawning (mean=4.5 sounds/min) (t=4.88, df=118, P>0.001). Within the spawning season, there was a significant increase in the rate of sound production on spawning than on nonspawning days (t=9.23, df=203, P>0.001), and peak rates occurred during spawning events (Fig. 5). Similarly, a significant increase in sound intensity was observed for sounds recorded on spawning days than on nonspawning days (t=1.89, df=438, P=0.030).

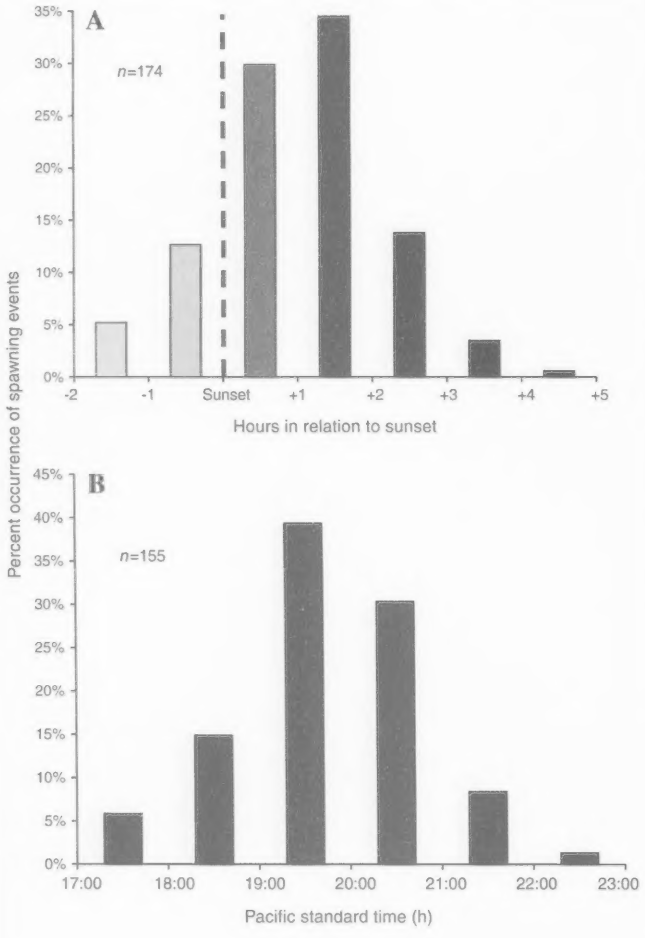

Figure 2

The percent occurrence of white seabass (*Atractoscion nobilis*) spawning events illustrating diel spawning periodicities (**A**) in relation to sunset and (**B**) according to Pacific Standard Time for 2001–03. Sample sizes correspond to the number of spawning events that could be assigned to an hourly interval. (A) Dashed line depicts the time of sunset and shading represents diminishing light levels throughout the evening. There is no shading in (B) because of seasonal changes in the time of civil twilight.

The basic sound types that have been identified for the white seabass are single pulse-trains, multiple pulse-trains, drum-rolls, thuds, and hydrodynamic booms (Fig. 6). A rapid succession of overlapping drum-roll and thud sounds resulted in recognizable spawning chants during the release of gametes. All sound types occurred on days of spawning; however, only hydrodynamic booms along with single and multiple pulse-trains of reduced rate and intensity occurred on days with no spawning (Fig. 7, A and B).

Single pulse-trains increased in rate and intensity on audio recordings that corresponded with spawning. The rate and intensity of pulse-trains remained high between successive spawning events and throughout the 1-h period after spawning.

Multiple pulse-trains occurred as a series of two to six pulse-trains, typically in a rhythmic pattern every 4–6 seconds. Weak multiple pulse-trains were detected throughout the day during the spawning season, but sound intensity greatly increased during the 90-min period after spawning events (Fig. 7B). Post-spawning multiple pulse-trains were of higher intensity than any other type of white seabass sounds.

Drum-rolls and thuds increased in rate and intensity during the initiation of spawning behavior and were not detected outside of the 4-h period surrounding spawning. Drum-rolls and thuds occurred in repetitive trains and were categorized as abbreviated chants or spawning chants when overlapping sounds became audibly indistinguishable.

Abbreviated chants consisted of rapid sequences of drum-rolls and thuds that terminated abruptly after 3–5 sec of spawning behavior and there was no release of gametes. Abbreviated chants were shorter in duration and lower in intensity than spawning chants and were

most commonly detected within 30 minutes before a spawning or in between successive spawning events.

Spawning chants were detected only during the release of gametes and consisted of an elongate series of intense, overlapping drum-roll and thud sounds at an average rate of 7 sounds/sec that continued for up to 55 seconds. The intensity of spawning chants was second only to multiple pulse-trains recorded after spawning.

Hydrodynamic booms were caused by sudden pressure waves generated underwater by rapid body movement and occurred throughout the day and night over the entire year. Their average rate and intensity remained relatively constant throughout the day and throughout the year, but increased considerably during feeding or in the presence of a predator around the net pens.

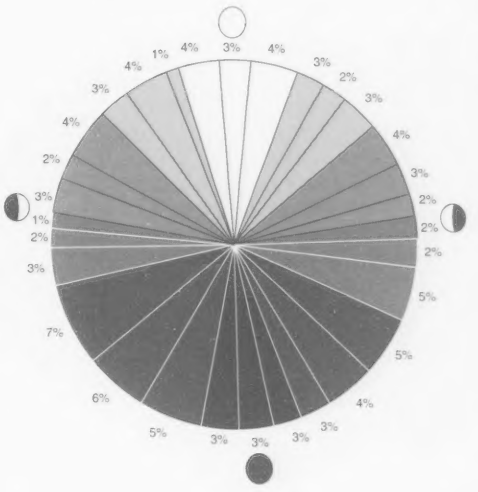

Figure 3

Lunar spawning periodicity of white seabass (*Atractoscion nobilis*) for 297 spawning events documented from 2001 through 2003. Values correspond to the % occurrence of spawning events on each day of a synodic month. Increased shading represents the lunar cycle as it moves from full (light) to new (dark). Spawning activity was not uniform over the lunar cycle (χ^2=49.57, df=29, P=0.01).

Discussion

Seasonal spawning

White seabass maintain a protracted 5-month spawning season from March through July, and 98% of spawning events are documented during this period. Allen and Franklin (1992) identified a peak in the catch per unit of effort of newly settled young-of-the-year white seabass off Southern California in July 1988 and June 1989, supporting the seasonal spawning data presented here. A March–July spawning season is a month earlier than the previously estimated April–August spawning season for white seabass (Skogsberg, 1939). Annual California Cooperative Oceanic Fisheries Investigation (CalCOFI) surveys revealed that white seabass larvae were most abundant off central Baja California from May through August and abundance peaked in July (Moser et al., 1983), which is later than the May peak in spawning described here. Latitudinal differences in photoperiod and water temperature throughout the southern extent of the population range may explain this discrepancy.

One-third of white seabass spawning activity occurred outside the current March 15–June 15 restricted commercial fishing period and time of reduced catch allowable under recreational bag limits. Under present regulations, spawning aggregations off California are exposed to heightened recreational and commercial fishing effort for approximately 60 days each season. Delaying the opening of commercial fishing season in addition to maintaining a one-fish recreational bag limit throughout the spawning season would considerably benefit white seabass spawning stocks.

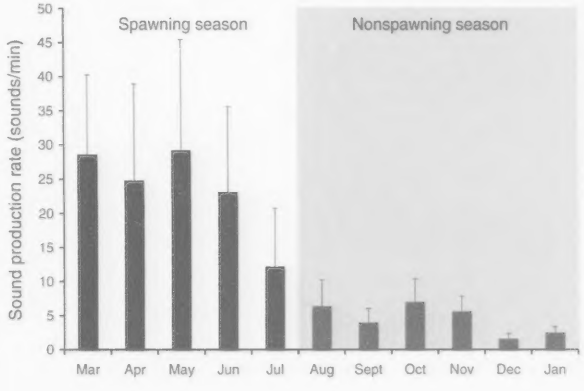

Figure 4

Mean monthly rate (±1 standard error) of all white seabass (*Atractoscion nobilis*) sounds for audio recordings (n=366) made from March 2003 to January 2004. A significant increase in sound production occurred during the spawning season (t=4.88, df=118, P>0.001). Hydrodynamic booms, which are generated by rapid movement through the water, accounted for a majority of the sounds recorded during the nonspawning season.

Diel spawning

A dusk and nighttime spawning period, as documented here for white seabass is common in other temperate sciaenids (Holt et al., 1985;

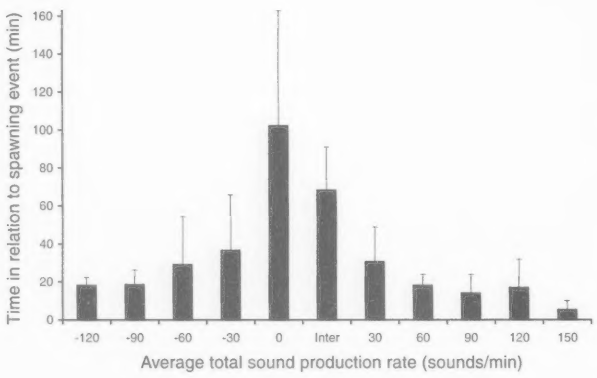

Figure 5

Mean sound production rate (±1 standard error) of white seabass (*Atractoscion nobilis*) for all combined sound types in relation to the release of gametes during 3-min evening recordings (n=102) when spawning was documented from March to July 2003. The term "Inter" refers to the period between successive spawning events. The time between successive spawning was 1 to 98 min (mean=31 min).

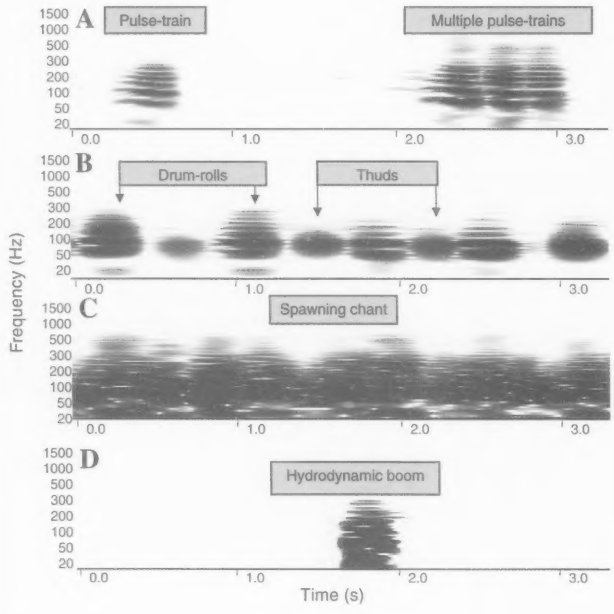

Figure 6

Three-second sonograms of five basic sound types (**A, B, D**) generated by white seabass (*Atractoscion nobilis*), in addition to an identifiable spawning chant (**C**) consisting of overlapping drum-roll and thud sounds generated during the release of gametes. Modified from Aalbers and Drawbridge (in press).

Taylor and Villoso, 1994) and in many fish species worldwide (Johannes, 1978). Spawning during low-light conditions may occur to reduce predation rates on both freshly spawned eggs and spawning adults (Lobel, 1978; Holt et al., 1985). Broadcast spawners may further benefit from synchronizing spawning activity by temporally concentrating reproductive effort and maximizing time allocated for diurnal movements and feeding.

Lunar spawning

White seabass spawned throughout the lunar cycle; although more individuals may have achieved reproductive readiness following the new moon, because 63% of mass spawning events occurred from the new moon until four days after the new moon. The majority of known lunar spawning periodicities occur around the new or full moon, possibly to reduce egg predation through increased dispersal around larger tidal flows or to ensure that males and females collectively achieve reproductive readiness (Johannes, 1978).

Environmental conditions

The majority of spawning occurred as water temperature and photoperiod increased during the spring and early summer; these factors appear to be important in stimulating white seabass spawning activity. Most spawning occurred at water temperatures between 15 and 18°C; this range is consistent with that observed under hatchery protocols to artificially induce spawning in broodstock tanks. Although more spawning was documented around high tide, this effect can be attributed to the increased occurrence of evening high tides during summer within the mixed semidiurnal tide regime of southern California (Flick, 2000).

Experimental variables

Although variations in the onset and duration of spawning seasons and the number of spawning events per season may represent biological responses to shifting environmental conditions (e.g., temperature changes), differences may be partly attributed to research procedure. The captive population was 34% lower in 2001 because 21 white seabass were not combined with the original 41 fish until after the 2001 spawning season. Additionally, increases in fish size and acclimation time over the 3-year period may have been responsible for the

46% increase in observed spawning activity in 2003 compared to 2001. Improved recognition of fish behaviors and patterns likely enhanced the detection of spawning events in 2002 and 2003. Difficulty in differentiating successive spawning events solely on the presence of eggs considerably reduced the total number of documented events, along with an inability to visually detect spawning after dark. Variations in white seabass feed rations may have influenced interannual spawning activity because condition factor influences fecundity in weakfish (Lowerre-Barbieri et al., 1996), haddock (*Melanogrammus aeglefinus*) (Hislop et al., 1978), and Atlantic cod (*Gadus morhua*) (Kjesbu and Klungsoyr, 1991).

Spawning frequency

Although data were not extensive enough to accurately determine individual white seabass spawning frequency or fecundity, it was apparent that females are serial spawners. The average number of observed spawning events per female in 2001, 2002, and 2003 was 2.0, 3.1, and 2.1, respectively; however, these values are underestimated because of experimental limitations. The same female spawned on 12 June 2002 between 2010 h and 2040 h and again on 16 June 2002 at 2038 h, indicating a capability for four-day spawning intervals.

The observed shift in the diel spawning periodicity in relation to sunrise and sunset as the spawning season progressed indicates that final oocyte maturation is not triggered solely by light intensity or the time of sunrise and sunset. White seabass exhibited a more consistent 24-h diel spawning pattern throughout the season, indicating that gamete development may occur with a circadian rhythm that could be modified through a combination of environmental cues (Taylor, 1984). Spawning rhythms and gamete development fluctuate synchronously with plasma estradiol-17β levels in female spotted seatrout and with testosterone and 11ketotestosterone concentrations in males (Brown-Peterson, 2003). In addition to regulating seasonal and diel spawning rhythms, increases in the levels of plasma androgens are correlated with seasonal hypertrophy of sonic musculature as male weakfish come into spawning condition (Connaughton and Taylor, 1994; Connaughton et al., 1997).

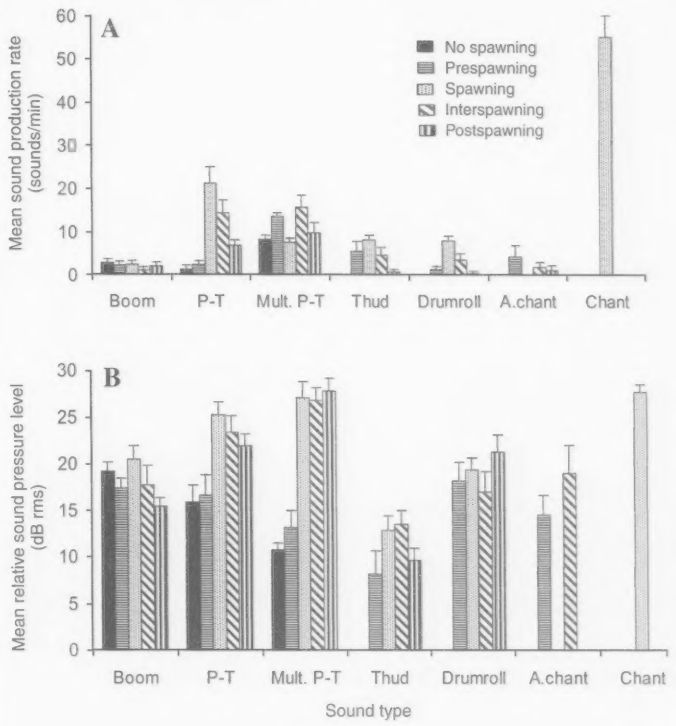

Figure 7

(**A**) The mean rates (±1 standard error) and (**B**) mean relative sound pressure levels (dB rms [root mean square] values do not represent absolute sound pressure level) of white seabass (*Atractoscion nobilis*) sounds for all 3-min evening recordings (n=151) made from March through July 2003. Recordings are grouped by period of occurrence in relation to the time of spawning or categorized as "no spawning" on evenings that spawning did not occur. Prespawning intervals spanned the 2-h period before initial spawning; postspawning intervals spanned the 2.5-h period after final spawning events; interspawning intervals spanned the period between successive spawning events, which occurred 1 to 98 minutes apart (mean=31 min). (P-T=pulse-train, Mult. P-T=multiple pulse-trains, A. chant = abbreviated chant, Chant=spawning chant).

Sound patterns

White seabass may experience alterations in reproductive endocrine hormones and physiological features that are similar to those observed in other sciaenids, because peak diel and seasonal spawning rhythms were directly correlated with increases in the rate, intensity, and variety of sound production. Sound production in orangemouth corvina and weakfish also culminates seasonally during peak spawning in May and diminishes in late July as spawning subsides (Fish and Cummings, 1972; Connaughton and Taylor, 1995). Additionally, diel increases in the sounds of weakfish and spotted seatrout have been documented after sunset during hours of

reproductive activity (Connaughton and Taylor, 1995; Gilmore, 2003).

The rate of white seabass sound production increased 20-fold during the release of gametes, establishing the highest rate and second highest intensity for all sounds. Measurable increases in the intensity of white seabass spawning chants and postspawning multiple pulse-trains were consistent with the audible detection of these sounds through the net-pen pipes and the hull of an adjacent boat. A chorus of orangemouth corvina sounds increased ambient noise within the Salton Sea by 50 dB during the spawning season (Fish and Cummings, 1972). All of the distinct white seabass sound types were present on audio recordings during spawning. In spotted seatrout, four major sound types were reported, all of which occurred during courtship and spawning (Gilmore, 2003).

Sound function

Direct correlations between spawning activity and sound production substantiate the hypothesis that white seabass sounds function to enhance reproductive success. Weak single and multiple pulse-trains that were audible throughout the spawning season may help white seabass maintain aggregations. Single pulse-trains before spawning may augment courtship behaviors or communicate reproductive readiness. Intense multiple pulse-trains after spawning may serve to attract surrounding females into spawning aggregations. Identifiable spawning chants recorded during actual spawning likely function to enhance reproductive success by synchronizing the release of gametes.

Conclusion

This study provides a strong correlation between verified white seabass spawning activity and sound production patterns. The noninvasive techniques used in this study can be extended to examine reproductive characteristics of other sound-producing fish species and alleviate difficulties associated with documenting spawning after dark. The essential fisheries information provided on key reproductive characteristics will help fisheries managers in designing strategies to sustain this economically important species and reduce the likelihood of another severe population decline. Baseline findings from this work can be extended to acoustically monitor white seabass spawning aggregations in order to investigate critical spawning habitat and to help determine better placement of marine protected areas throughout California.

Acknowledgments

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Abstract—King mackerel (*Scomberomorus cavalla*) are ecologically and economically important scombrids that inhabit U.S. waters of the Gulf of Mexico (GOM) and Atlantic Ocean (Atlantic). Separate migratory groups, or stocks, migrate from eastern GOM and southeastern U.S. Atlantic to south Florida waters where the stocks mix during winter. Currently, all winter landings from a management-defined south Florida mixing zone are attributed to the GOM stock. In this study, the stock composition of winter landings across three south Florida sampling zones was estimated by using stock-specific otolith morphological variables and Fourier harmonics. The mean accuracies of the jackknifed classifications from stepwise linear discriminant function analysis of otolith shape variables ranged from 66–76% for sex-specific models. Estimates of the contribution of the Atlantic stock to winter landings, derived from maximum likelihood stock mixing models, indicated the contribution was highest off southeastern Florida (as high as 82.8% for females in winter 2001–02) and lowest off southwestern Florida (as low as 14.5% for females in winter 2002–03). Overall, results provided evidence that the Atlantic stock contributes a certain, and perhaps a significant (i.e., ≥50%), percentage of landings taken in the management-defined winter mixing zone off south Florida, and the practice of assigning all winter mixing zone landings to the GOM stock should be reevaluated.

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Spatial and temporal variability in the relative contribution of king mackerel (*Scomberomorus cavalla*) stocks to winter mixed fisheries off South Florida

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King mackerel (*Scomberomorus cavalla*) are large coastal pelagic scombrids distributed from Massachusetts to Brazil in the western Atlantic Ocean, including the Caribbean Sea (McEachran and Fechhelm, 2005). They support important commercial and recreational fisheries throughout the U.S. Gulf of Mexico (GOM) and in the Atlantic Ocean (Atlantic) off the southeastern United States. King mackerel currently are managed in U.S. waters as two migratory groups, one resident in the GOM and one off the southeast U.S. coast. The two-stock migratory group or two-stock model of population structure was adopted in the early 1980s based on tagging data indicating fish from the respective regions had distinct migratory pathways (Sutter et al., 1991). Subsequent studies demonstrated growth differences (DeVries and Grimes, 1997) and genetic distinctiveness (Gold et al., 1997, 2002) between the stocks.

Assessment and management of U.S. king mackerel stocks is complicated because of the seasonal mixing between GOM and Atlantic fish. Mark-recapture (Sutter et al., 1991) and catch-per-unit-of-effort studies (Trent et al., 1987) have indicated that winter migrations of king mackerel occur from both the eastern GOM and Atlantic to south Florida where the mixed stock is targeted by a winter fishery. Thus, an area that stretches from the Volusia-Flagler county line in northeast Florida to the Monroe-Collier county line in southwest Florida was defined in the early 1980s by the Gulf of Mexico and South Atlantic Fishery Management Councils as a mixing zone between the two stocks (GMFMC and SAFMC, 1985; Fig. 1). From April to October, all king mackerel landings in the mixing zone are attributed to the Atlantic stock, and landings from November through March are attributed to the GOM stock. This

somewhat subjective stock assignment system was implemented in an effort to protect the GOM migratory group, which was estimated to be overfished. However, assessment and management are complicated by the presence of both Atlantic and GOM fish in the mixing zone during winter.

Accurate estimation of the contribution of each stock to winter landings is necessary for effective management and conservation. Several different techniques to distinguish these two groups have been explored in various studies. Tagging studies support the current two-stock management approach but have not resolved winter inter-stock mixing proportions. Likewise, although studies of population genetics have confirmed that genetically distinct Atlantic and GOM stocks exist, genetic divergence between the two stocks is weak; thus differences are not robust enough to distinguish winter landings effectively (Broughton et al., 2002; Gold et al., 2002).

Analysis of otolith shape has proven to be a useful technique for stock discrimination in several marine teleosts (e.g., Atlantic cod, *Gadus morhua* [Campana and Casselman, 1993]; Atlantic salmon, *Salmo salar* [Friedland and Reddin, 1994]; and haddock, *Melanogrammus aeglefinus* [Begg et al., 2001]). DeVries et al. (2002) demonstrated that otolith-shape parameters effectively distinguish Atlantic and GOM female king mackerel; classification accuracies from linear discriminant function models ranged from 65.8% to 85.7%. They applied otolith-shape variables as natural markers to estimate the stock identity of female king mackerel landed between Cape Canaveral and West Palm Beach, Florida, in winter 1996–97. A maximum likelihood model parameterized with stock-specific otolith-shape data revealed that Atlantic fish represented 99.8% of the composition of winter mixed fishery landings, thus casting doubt on the management practice of attributing all winter mixing zone landings to the GOM stock.

The objective of this study is to employ otolith-shape analysis to examine temporal and spatial variability in the Atlantic and GOM stock contribution to king mackerel landings around during winter around the southern tip of Florida. We build on the earlier success of DeVries et al. (2002) by examining sex-specific differences in otolith shape and by estimating the contribution of both Atlantic males and females to landings in the winter mixing zone. Temporal and spatial variability in stock mixing also is examined by estimating the Atlantic stock contribution to landings in three south Florida sampling zones distributed across the winter mixing area during two consecutive winters.

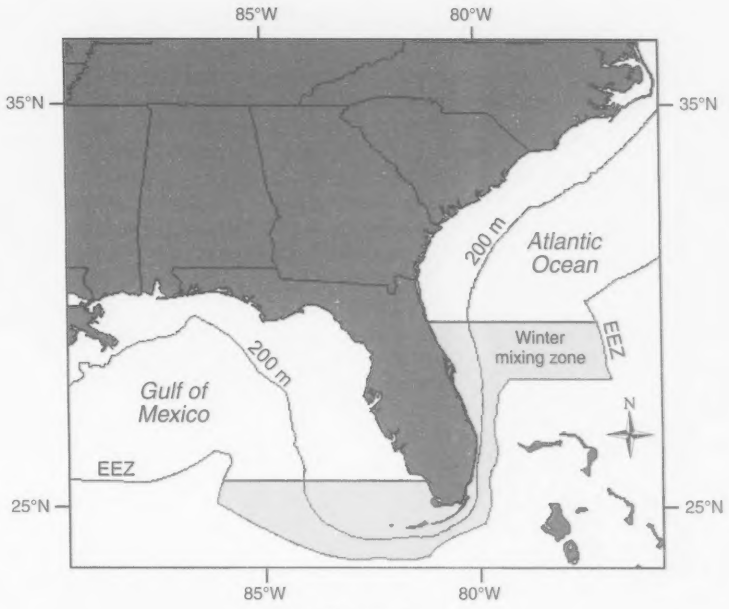

Figure 1

Winter mixing zone established for king mackerel (*Scomberomorus cavalla*) off south Florida. The zone exists throughout the U.S. exclusive economic zone (EEZ) but fish are mostly found over the shelf (200-m isobath). All landings taken from the zone from November through March are attributed to the Gulf of Mexico stock. During the rest of the year, landings are attributed to the Atlantic stock.

Materials and methods

King mackerel were sampled from recreational landings caught in eastern GOM and U.S. south Atlantic waters from April to October 2001 and 2002 when stock distributions did not overlap (Fig. 2); nearly all samples came from summer (June through September) months. Fish were measured to the nearest cm fork length (FL) and sex was determined by macroscopic examination of gonads. When possible, both sagittal otoliths were removed from fish, but for some samples only one sagitta was available. Once extracted, otoliths were cleaned of adhering tissue and placed in plastic vials for storage. Age of fish was estimated by examining whole otoliths for fish less than 80 cm FL and thin sections were prepared for aging larger fish (DeVries and Grimes, 1997). Stratified random sampling was employed once all samples were aged to select up to 15 fish from each of five age categories: ages 2, 3, 4, 5, and 6 years. This age range was selected because winter landings typically are of small, young fish.

King mackerel also were sampled from commercial and recreational landings from three different zones

off south Florida from December 2001 to March 2002 and December 2002 to March 2003 (Fig. 2). Zone 1 represented southwest Florida and primarily consisted of samples from the commercial gillnet fishery near and to the east of the Dry Tortugas. Zone 2 represented south central Florida and consisted of samples from the recreational charter boat fishery operating south of Islamorada in the Florida Keys. Zone 3 represented southeast Florida and primarily consisted of samples from the commercial troll fishery from Sebastian Inlet to south of West Palm Beach, Florida. Collection and aging procedures for winter fish otoliths followed the same protocol as summer sampling.

Left sagittal otoliths were digitized sulcus side down with an image analysis system running Image-Pro image analysis software (vers. 4.5, Media Cybernetics Inc., Bethesda, MD). Otolith samples were magnified by 13× with a dissecting microscope before their images were captured with the image analysis system. When left otoliths were damaged or unavailable, right otoliths were digitized and their mirror images were used for shape analysis (DeVries et al., 2002). The autotrace feature in Image Pro then was used to trace the posterior surface of the otolith. Otolith tracing began at the tip of the antirostrum, was directed manually across the base of the rostrum, and then the software traced the posterior portion of the otolith. Thus, rostra were excluded from otolith-shape analysis because the anterior rostrum is fragile and often was broken during otolith collection (DeVries et al., 2002).

Fourier coefficients were computed with an algorithm within Image-Pro, and we used the mathematically determined centroid as the center of an otolith. The Image-Pro algorithm used 128 vectors at equally spaced polar angles to create an accurate picture of the otolith outline. The amplitudes of the first 20 Fourier harmonics were calculated for analysis because each additional harmonic provides increasingly finer detail of the otolith outline. For example, 97–99% of otolith-shape variability in haddock (*Melanogrammus aeglefinus*) is contained in the first ten harmonics (Begg and Brown, 2000). Fourier amplitudes were standardized to remove the effect of otolith size by dividing each amplitude by the mean radial length of the otolith. In addition to the first 20 standardized Fourier harmonics, the Image-Pro software calculated otolith area, perimeter, rectangularity, circularity, and roundness for a total of 25 shape variables. All variables were tested for univariate normality with the Shapiro-Wilks statistic and for homogeneity of variance with an F_{max} test. Transformations were necessary for perimeter (natural log) and Fourier harmonics 13–16 (square-root) in order to meet parametric statistical analysis assumptions of normality and homogeneity of variances.

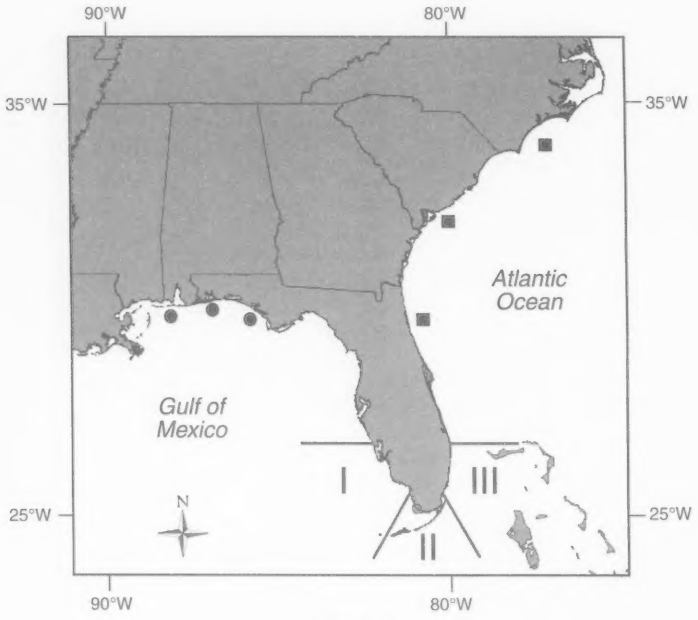

Figure 2

Map of sampling locations for king mackerel (*Scomberomorous cavalla*) in summers 2001 and 2002 in U.S. Atlantic Ocean waters (squares) and the Gulf of Mexico (circles). The map also shows the three winter sampling zones around southern Florida from which fish were sampled in winter 2001–02 and 2002–03 for estimates of the Atlantic stock contribution to winter landings.

Ontogenetic effects on otolith shape were tested by computing the correlations of shape variables with fish length. Ontogenetic effects were removed from each shape variable that was significantly correlated with fish length by subtracting the slope of the least squares linear relationship between length and a given variable. Slope-corrected data were used in all subsequent analyses.

Multivariate analysis of variance (MANOVA) was performed to test for potential shape differences between sides in a subset of 50 left and right sagittal otolith pairs (SAS, vers. 6.11, SAS Inst., Inc., Cary, NC). A second MANOVA also was performed to test for stock-specific differences in summer samples. The effect of other factors, including sex, age, and sampling year, on otolith shape parameters also was tested within this second MANOVA.

Stepwise linear discriminant function (LDF) analysis was performed separately for sexes and years on otolith-shape variables from summer sampled fish with the PROC STEPDISC procedure in SAS. The LDF procedure selected variables that were effective predictors of stock identity. Jackknife cross-validation was used to evaluate the performance of resultant discriminant functions. Classification suc-

cess was estimated as the percentage of individuals correctly classified to stock.

The contribution of the Atlantic stock to winter fishery landings in each winter sampling zone was estimated by using the maximum likelihood (ML) modeling approach described in DeVries et al. (2002). Mixing estimates were calculated for males and females separately by sample year. Otolith-shape variables were used in a two-step expectation-maximization (EM) algorithm written for the S-Plus statistical package (Insightful Corp., Seattle, WA) (Millar, 1987; DeVries et al., 2002). Sex- and year-specific ML models first were parameterized with otolith-shape data from summer-sampled fish. Then, the EM algorithm computed estimates of the percentage of landings within a given winter sampling zone that were members of the Atlantic stock based on their otolith shape parameters. A bootstrap procedure (n=500 bootstraps) was used to compute bias-corrected ninety percent confidence intervals around the maximum likelihood estimate (MLE) of Atlantic stock contribution.

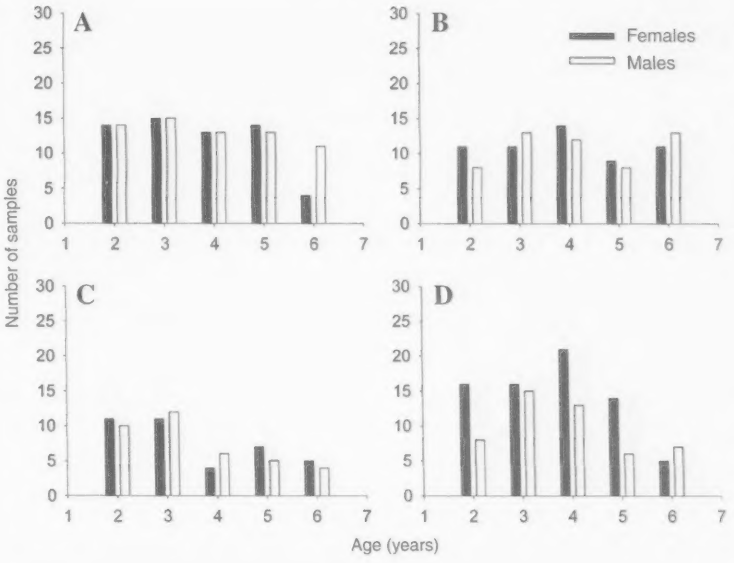

Figure 3

Age distribution of king mackerel (*Scomberomorus cavalla*) in samples collected in summers 2001 and 2002. (**A**) Atlantic 2001; (**B**) Atlantic 2002; (**C**) Gulf of Mexico (GOM) 2001; and (**D**) GOM 2002.

Results

Summer sample sizes differed somewhat between stocks, sexes, and sampling years. One hundred twenty-six king mackerel (60 females, 66 males) were sampled in summer 2001, and 110 fish (56 females, 54 males) were sampled in summer 2002 from Atlantic waters. Seventy-three fish (37 females, 36 males) were sampled in summer 2001, and 120 fish (71 females, 49 males) were sampled in summer 2002 from the GOM. The age distributions of summer-sampled king mackerel generally were similar between sexes, migratory groups, and years (Fig. 3).

Sex-specific sample sizes were more variable from south Florida sampling zones during winter than during summer. In winter 2001–02, 153 fish (85 females, 68 males) were sampled in zone 1, 50 fish (44 females, 6 males) were collected in zone 2, and 142 fish (67 females, 75 males) were sampled in zone 3. In winter 2002–03, 158 fish (85 females, 73 males) were collected in zone 1, 72 fish (50 females, 22 males) were collected in zone 2, and 153 fish (86 females, 67 males) were collected in zone 3. The age distributions of winter-sampled king mackerel were skewed toward younger fish in comparison to summer samples (Fig. 4).

Correlation analysis indicated some ontogentic effects on otolith shape may have been present. Several shape variables were significantly correlated with fish length (area, perimeter, roundness, rectangularity, circularity, Fourier harmonics 1–9, 11–14, 17, 19, and 20); the method described above was applied to remove the correlation of those variables with respect to fish length. MANOVA results indicated there were no significant differences in otolith shape between left otoliths and right otoliths (MANOVA, P<0.601).

Morphological features of otoliths proved to be different between stocks, but several other factors also significantly affected otolith shape. Sex and age, as well as stock, significantly affected otolith shape (MANOVA, P<0.001), but sampling year did not (MANOVA, P=0.964). Six of 25 shape variables were significantly different between sexes (ANOVA, P<0.05). Most of the shape differences were in variables that described gross otolith morphological features (area, perimeter, roundness, circularity, and rectangularity), and only one of the significantly different variables was a Fourier harmonic. Twelve of 25 shape variables were significantly different among ages (ANOVA, P<0.05), and most of the differences were in Fourier harmonics. Nine of 25 shape variables were significantly different between stocks (ANOVA, P<0.05). Most of the stock-specific shape differences

were in gross otolith morphological features or low-order Fourier harmonics.

Sex and year-specific linear discriminant functions yielded a range of shape variables selected, and the mean accuracy of classifications ranged from 65.8% to 76.4% among models (Table 1). Discriminant functions included between five and seven variables. The highest classification accuracies from a given model were 71.1% for GOM females and 81.7% for Atlantic females in 2001 (mean accuracy 76.4%). The lowest classification accuracies were 61.2% for GOM males and 70.4% for Atlantic males in 2002 (mean accuracy 65.8%). Classification accuracies were slightly higher for Atlantic fish (67.9–81.7%) than for GOM fish (61.2–71.1%) for most models.

Atlantic stock king mackerel contributed to landings in all three winter sampling zones. Maximum likelihood models estimated that the contribution of Atlantic fish to winter landings ranged from 14.5% for females in zone 1 in 2002 to 99.9% for males in zone 2 in 2001 (Table 2). Bias-corrected bootstrapped 90% confidence intervals varied among zones and between years but generally were on the order of point estimates ±20%. Bootstrap cumulative frequency distributions demonstrated that although the majority of bootstraps fell near point estimates, wide confidence intervals resulted from long upper and lower distribution tails (Figs. 5 and 6).

The estimated contribution of the Atlantic stock to 2001–02 winter landings was similar between males and females among all three winter sampling zones, except for zone 2 where few males were sampled (Table 2). In winter 2002–03, Atlantic females contributed less than males and also had lower contribution estimates than females in 2001–02. Atlantic males had similar contribution estimates during both sampling years. Overall, a gradient in contribution estimates was observed; there were higher Atlantic stock percentages in southeast Florida (zone 3) and declining Atlantic stock presence in southwest Florida landings (zone 1).

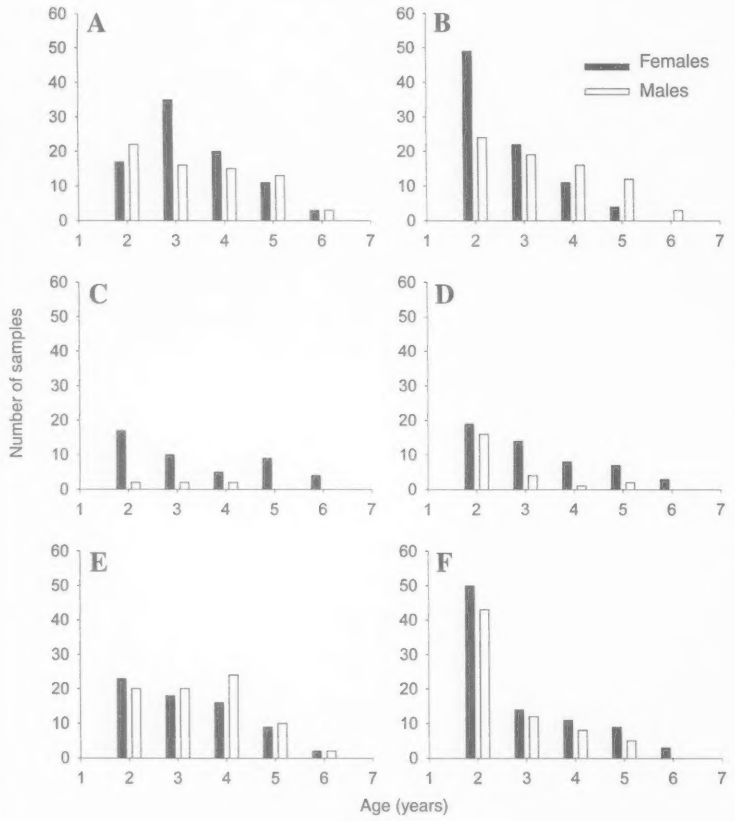

Figure 4

Age distribution of king mackerel (*Scomberomorus cavalla*) in samples collectged in winters 2001–02 and 2002–03. (**A**) = 2001 zone 1 ; (**B**) = 2002 Zone 1 ; (**C**) = 2001 zone 2 ; (**D**) = 2002 zone 2 ; (**E**) = 2001 zone 3 ; and (**F**) = 2002 zone 3.

Discussion

Classification accuracies from stepwise linear discriminant function analysis confirm the feasability of using otolith-shape parameters to distinguish king mackerel stocks but also demonstrate that stock-specific otolith-shape parameters provide natural tags that are far from perfect (i.e., <=100% stock discrimination success). The classification success that we report (61.2% to 81.7%) is similar to the range reported in shape-based stock or population discrimination for other fishes (e.g., 54.9% to 89.3% for lake whitefish, *Coregonus clupeaformis* [Casselman et al., 1981]; 63.9% to 87.5% for Atlantic salmon [Friedland and Reddin, 1994]; 61% to 83% for haddock [Begg et al., 2001]; and, 63.6% to 83.3% for coral trout, *Plectropomus leopardus* [Bergenius et al., 2006], as well as that previously reported by DeVries et al. [2002] for female king mackerel [65.8% to 85.7%]). However, the lack of more distinct differences in otolith shape between stocks likely contributed significantly to the wide confidence intervals estimated

Table 1

Accuracies of jackknifed classifications from stepwise linear discriminant function models computed with otolith shape parameters to estimate summer king mackerel (*Scomberomorus cavalla*) stock identity. The model column identifies which sex- and year-specific models are examined. Numbers in the parameters column represent Fourier harmonics; *Ro* = Roundness, *Re* = Rectangularity, and *P* = Perimeter. Remaining columns indicate the percentage of fish correctly classified to the Atlantic and Gulf of Mexico (GOM) stocks with the jackknife algorithm.

Model	Parameters	Accuracy (%) for Atlantic region	Accuracy (%) for GOM region	Mean accuracy (%)
Females 2001	*Ro*, *Re*, 3, 7, 20	81.7	71.1	76.4
Males 2001	3, 5, 6, 8, 9, 10	69.7	67.6	67.8
Females 2002	*P*, *Ro*, 2, 9, 13, 15, 16	67.9	70.8	69.4
Males 2002	*P*, *Re*, 2, 8, 11, 13	70.4	61.2	65.8

Table 2

Maximum likelihood estimates (MLE) of the contribution (%) of Atlantic stock king mackerel (*Scomberomorus cavalla*) to winter landings in each of three south Florida winter sampling zones by sex and year, with 90% bias-corrected confidence intervals (CI) provided. The model column indicates which zone and year was estimated.

Model	MLE females	90% CI	MLE males	90% CI
Zone 1, 2001–02	61.0	32.2–82.7	61.0	40.2–73.9
Zone 2, 2001–02	48.6	20.1–67.2	99.9	60.9–100.0
Zone 3, 2001–02	82.8	62.8–99.8	76.0	57.0–99.7
Zone 1, 2002–03	14.5	0.0–28.9	45.0	21.2–70.0
Zone 2, 2002–03	41.3	20.9–68.9	83.1	49.4–100.0
Zone 3, 2002–03	40.4	24.2–59.5	71.9	51.5–99.4

from bootstrapped MLEs of Atlantic stock contribution to south Florida winter king mackerel landings. Imprecision in those estimates prohibits more definitive conclusions about the relative contribution of GOM and Atlantic stocks to winter fisheries off south Florida. Nonetheless, it is possible to infer from our results that the Atlantic stock contributes substantially more than the zero percent of winter south Florida landings that is currently assumed by fishery management groups.

Most of the otolith-shape differences between king mackerel stocks were observed in gross morphological variables and low-order Fourier harmonics. Low-order Fourier harmonics are related to general otolith shape, whereas high-order Fourier harmonics are related to increasingly fine-scale variation (Bird et al., 1986). DeVries et al. (2002) reported that gross otolith morphological parameters and low-order Fourier harmonics are significant contributors to otolith-shape variability in female king mackerel in southwest Florida, but they also reported many high-order Fourier harmonics to be significant as well.

Sex effects on king mackerel otolith shape were significant for every gross morphological variable but for only one Fourier harmonic; this results indicates that sex-specific shape differences exist at a general level. Sex effects are not surprising given that sexually dimorphic growth occurs in king mackerel; females achieve higher growth rates than males (Johnson et al., 1983; Manooch et al., 1987; Sturm and Salter, 1989; DeVries and Grimes, 1997). DeVries et al. (2002) examined only female king mackerel as a precaution against potential sex effects due to sexually dimorphic growth observed in this species. Most otolith shape studies that have tested for sex effects have found no significant differences between males and females (Bird et al., 1986; Castonguay et al., 1991; Bolles and Begg, 2000; Begg et al., 2001). In studies where sex effects were significant, other factors were deemed more influential (Campana and Casselman, 1993).

Otolith-shape variables in the models that best classified king mackerel migratory groups were not consistent between sampling years. This result indicates that new shape-analysis models should be developed each summer and used only to estimate the migratory group composition of landings of the next winter. It is unclear why parameters in a discriminant function model may be important one year but of little value in distinguishing stocks the next year. However, interannual variability in growth rates between stocks may explain why LDFs do not perform well from one year to

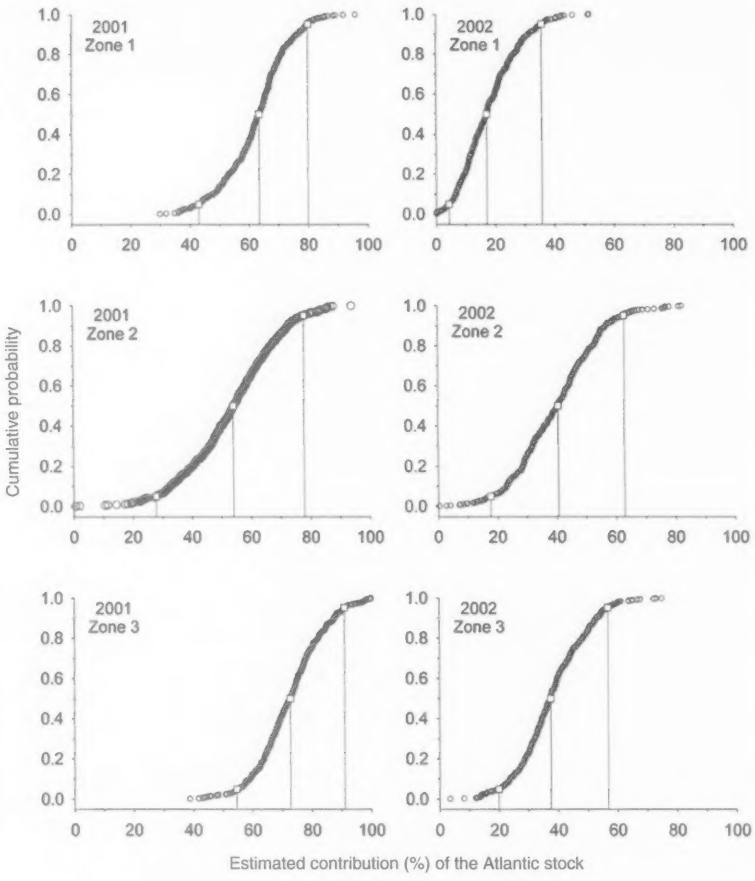

Figure 5

Cumulative probability distributions of bootstrapped estimates of the contribution of Atlantic stock female king mackerel (*Scomberomorus cavalla)* to landings at three south Florida sampling zones. Drop-lines indicate the 5th, 50th, and 95th percentiles of bootstrap distributions.

the next (Campana and Casselman, 1993). For example, cohort-specific discriminant function models computed for coral trout sampled on the Great Barrier Reef did a poor job distinguishing fish from another cohort to sampling region (34.3% to 39.7% classification success), a percentage that Bergenius et al. (2006) attributed to differences in growth rates ultimately caused by variability in oceanographic conditions.

Maximum likelihood estimates indicated that some percentage of winter landings in all three zones originated from the Atlantic stock in both study years. However, bootstrapped confidence intervals indicated considerable imprecision around point estimates. Cumulative probability distributions of bootstraps (n=500) were broad for females and males in both study years. However, even at the lower end of the confidence intervals, Atlantic fish were estimated to have contributed greater than 20% of landings in all three zones, except for females sampled in zone 1 during winter 2002–03.

Results potentially indicate that a distribution gradient may exist; more Atlantic king mackerel may contribute to landings from the Atlantic side (zone 3) and fewer Atlantic stock king mackerel contribute toward the GOM (zone 1). Mixing estimates for zone 2 are somewhere in the middle, with the exception of zone 2 males in 2001–02. However, the sample size of king mackerel in zone 2 in 2001–02 generally was low, particularly for males, and this shortage could account for the higher estimate for the Atlantic stock contribution.

Atlantic male and female king mackerel appear to have had similar contributions across all three south Florida sampling zones in winter 2001–02, but this

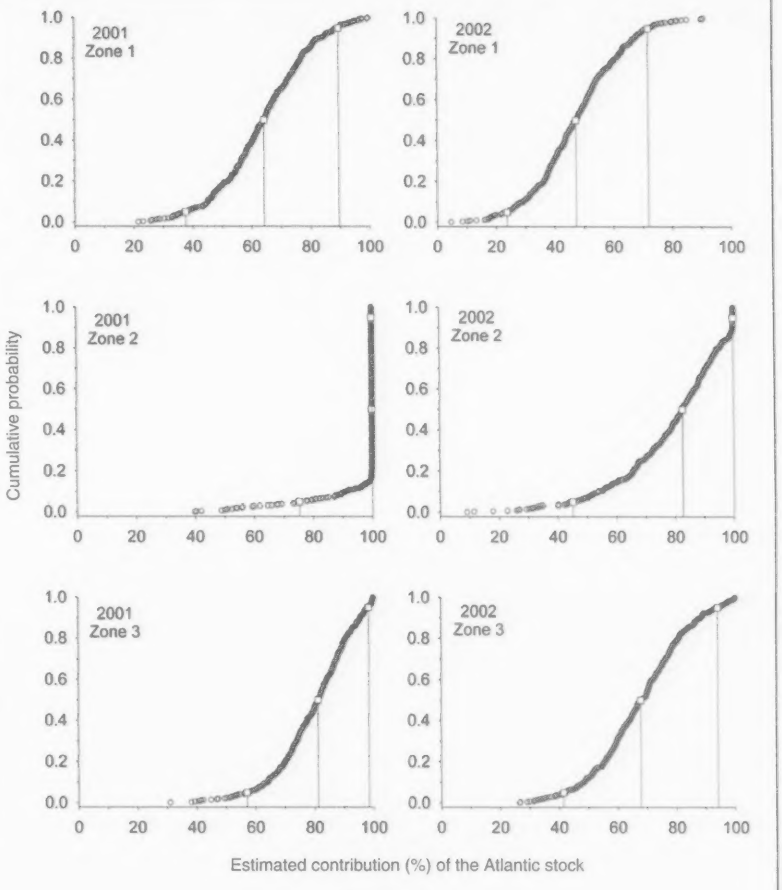

Figure 6

Cumulative probability distributions of bootstrapped estimates of the contribution of Atlantic stock male king mackerel (*Scomberomorus cavalla*) to landings at three south Florida sampling zones. Drop-lines indicate the 5th, 50th, and 95th percentiles of bootstrap distributions.

was not the case in winter 2002–03. Zone 1 and zone 3 in particular showed reductions of 35% and 45%, respectively, in the contribution of Atlantic females in 2002–03. It is unclear why Atlantic females were estimated not to have contributed as significantly to landings in these zones. Differences in classification accuracies between summer 2001 and summer 2002 females may have affected landings contribution estimates, but discriminant function classification accuracies differed by only 7% between years. The reduced contribution of Atlantic females in winter 2002–03 most likely reflects temporal variability in stock mixing.

Overall, results of this study provide further evidence that the U.S. Atlantic king mackerel stock contributes a certain, and perhaps a significant, percentage of landings taken in the management-defined winter mixing zone off south Florida. Based on our results, fisheries managers should consider adopting some form of a gradient approach in attributing south Florida winter landings to GOM and Atlantic stocks. An alternative, and perhaps more easily defended, management approach may be to assign 50% of winter mixing zone landings to the Atlantic stock in the absence of annual estimates of stock-specific landing contributions.

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Ichthyoplankton community in the Columbia River plume off Oregon: effects of fluctuating oceanographic conditions

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Abstract—Ichthyoplankton samples were collected at approximately 2-week intervals, primarily during spring and summer 1999–2004, from two stations located 20 and 30 km from shore near the Columbia River, Oregon. Northern anchovy (*Engraulis mordax*) was the most abundant species collected, and was the primary species associated with summer upwelling conditions, but it showed significant interannual and seasonal fluctuations in abundance and occurrence. Other abundant taxa included sanddabs (*Citharichthys* spp.), English sole (*Parophrys vetulus*), and blacksmelts (Bathylagidae). Two-way cluster analysis revealed strong species associations based primarily on season (before or after the spring transition date). Ichthyoplankton abundances were compared to biological and environmental data, and egg and larvae abundances were found to be most correlated with sea surface temperature. The Pacific Decadal Oscillation changed sign (from negative to positive) in late 2002 and indicated overall warmer conditions in the North Pacific Ocean. Climate change is expected to alter ocean upwelling, temperatures, and Columbia River flows, and consequently fish eggs and larvae distributions and survival. Long-term research is needed to identify how ichthyoplankton and fish recruitment are affected by regional and large-scale oceanographic processes.

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The Columbia River and its plume form a biologically rich environment. It is the largest river flowing into the Pacific Ocean in North America (Sherwood et al., 1990), has relatively large salmon runs, a healthy white sturgeon (*Acipenser transmontanus*) population, and probably the largest American shad (*Alosa sapidissima*) run in the world. The Columbia River estuary is also one of the largest estuaries on the west coast (Emmett et al., 2000) and supports abundant piscivorous marine mammal and bird populations (NMFS, 1997; Collis et al., 2002). Important marine fisheries located in the Columbia River plume include those for the following species: Dungeness crab (*Cancer magister*), salmon (*Oncorhynchus* spp.), and Pacific sardine (*Sardinops sagax)* (Emmett et al., 2005, 2006).

River plumes and their associated fronts are important habitats for fishes during their larval stages (Grimes and Funucane, 1991; Govoni and Grimes, 1992; Govoni, 1997). Large freshwater discharges from the Columbia River create abrupt oceanographic fronts that may be important for salmon (Schabetsburger et al., 2003; DeRobertis et al., 2005) and other fish species, and they concentrate some species of zooplankton (Morgan et al., 2005). The Columbia River plume has also been shown to be an important spawning and rearing habitat for some fishes (Richardson, 1981; Doyle et al., 1993, 2002; Doyle, 1995; Emmett et al., 1997), particularly for the northern stock of northern anchovy (*Engraulis mordax*) (Richardson, 1981). This region also has regular upwelling, which is a dominant feature of the California Current system and in the Columbia River plume region, and it ultimately determines the amount of primary, and probably secondary, production within this system (Hickey and Banas, 2003).

Ichthyoplankton surveys have been conducted to augment available abundance data on many commercially important fish species and are often used to determine spawning biomass (Lasker, 1985; Hunter and Lo, 1993) as well as to understand recruitment processes (Bailey, 1981; Cowan and Shaw, 2002). Data sets generated by such ichthyoplankton investigations provide an opportunity to determine the role that oceanographic processes and climate change play in recruitment variability of commercially valuable species.

There have been relatively few ichthyoplankton surveys conducted off the Pacific Northwest. In previous ichthyoplankton surveys conducted off Oregon, Richardson and Pearcy (1977), Brodeur et al. (1985), and Auth and Brodeur (2006) identified the ichthyofaunal community, but these surveys were relatively short in duration and the area near the Columbia River was not sampled. Other coastal ichthyoplankton surveys

have been conducted off the Columbia River, but have been limited to one year (Waldron, 1972) and thus it was not possible to identify any relationship between fluctuations in ocean conditions and changes in the ichthyofaunal community. However, Doyle (1995) did extensive sampling during spring (April–May), and some during other months from 1980–85 and 1987, and found distinct changes in the Northwest ichthyoplankton community resulting from the 1983 El Niño. Later, Doyle et al. (2002) examined ichthyoplankton in the Northeast Pacific, including the Pacific Northwest and the Columbia River plume, and identified regional, onshore, and offshore differences in the ichthyoplankton communities. Northern anchovy was a dominant species off the Pacific Northwest during all these ichthyoplankton surveys. Northern anchovy eggs and larvae have been found to be abundant around the Columbia River plume (Richardson, 1981; Emmett et al., 1997). However, detailed information on the temporal and annual variability of the dominant ichthyoplankton components is lacking. This is noteworthy, given the importance of larval and postlarval fishes in the diets of salmon in the plume region (Schabetsberger et al., 2003; DeRobertis et al., 2005) and the important role that forage fishes, particular northern anchovy, Pacific herring (*Clupea pallasi*), and smelt, play in the diet of piscivorous fishes, mammals, and birds in this region. Our study was initiated to provide data to address this need, and to further elucidate the early life histories of fish species that use the Columbia River plume under a variety of environmental conditions.

Repeated plankton sampling at two stations just south of the mouth of the Columbia River over a 6-year period provided us an opportunity to study interannual and seasonal changes in the diversity and species assemblages in the nearshore ichthyoplankton community of this region. We also identified the effects of fluctuating river flows and nearshore oceanic environmental conditions on the ichthyofaunal community. We were provided this opportunity because of the fortuitous overlap of our research with the regime change in the Pacific Decadal Oscillation (Mantua et al., 1997) in late 2002 (Goericke et al., 2005).

Materials and methods

Study site

The Columbia River has a diverse drainage basin that covers approximately 670,000 km^2 and has average flows of approximately 10,000 m^3/s the latter of which create a surface lens, or plume, of low-salinity water in the adjacent ocean that can extend up to 400 km offshore (Barnes et al., 1972). During summer, the plume flows outward and southward, but during winter it is confined to a narrow band along the Washington coast (Anderson, 1972; Hickey and Banas, 2003). In summer months the plume is characterized by lower salinities (<32 psu), higher turbidities, and higher temperatures than the corresponding variables for surrounding waters and is an important habitat for many species of invertebrates and fishes (DeRobertis et al., 2005; Morgan et al., 2005; Emmett et al., 2006).

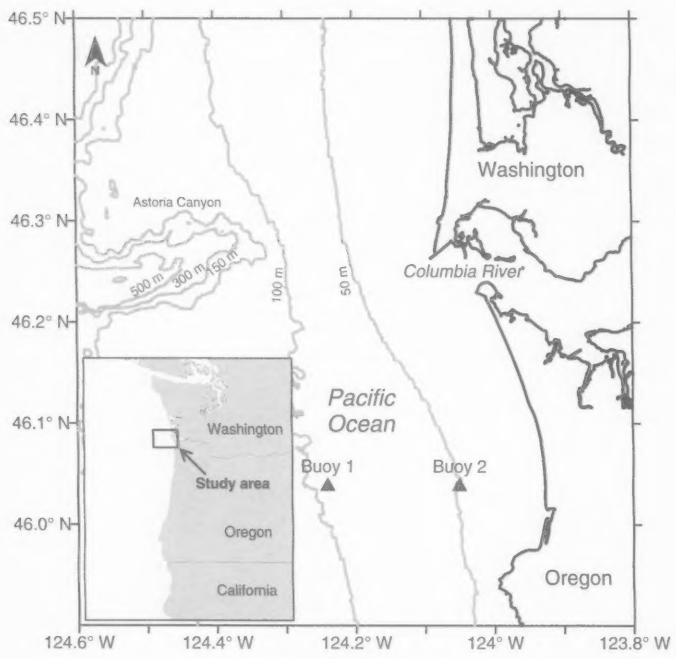

Figure 1

Map of the study area showing locations of two buoys off the Columbia River, Oregon, where ichthyoplankton was collected during survey cruises of the National Oceanic and Atmospheric Administration, National Marine Fisheries Service, 1999–2004. Also shown are depth contours (50–500 m) and the location of Astoria Canyon.

Data collection

Plankton samples were collected at two stations approximately every two weeks during the spring and summer, as well as occasionally during fall and winter in 1999–2004 (Table 1). The first station, buoy 1, is located 30 km from the mouth of the river and has a bottom depth of 98 m. The second station, buoy 2, is located 20 km from the mouth of the river and has a bottom depth of 47 m (Fig. 1). Oblique plankton tows to a depth of approximately 40 m were conducted with a 1-m diameter net with 335-μm mesh and a centrally mounted flow meter. The net was washed with seawater after each tow, and the contents were preserved in a 10% buffered formalin in seawater solution. Any large scyphomedusas captured in the net were rinsed and removed from the sample. A Niskin

Table 1

Total number (by year and month) of ichthyoplankton samples collected off the Columbia River, Oregon, during survey cruised conducted by the National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

	1999	2000	2001	2002	2003	2004	Total
January	2	0	0	0	0	0	2
February	0	0	1	0	0	0	1
March	2	2	0	0	0	0	4
April	3	1	0	2	2	2	10
May	4	3	4	1	6	6	24
June	2	2	6	3	6	4	23
July	2	2	5	2	4	4	19
August	0	0	0	0	0	0	0
September	1	0	0	0	0	0	1
October	0	0	0	0	0	0	0
November	0	0	0	0	0	0	0
December	1	0	0	0	0	0	1
Total	17	10	16	8	18	16	85

bottle was used to collect a water sample for chlorophyll analysis at the 3-m depth at each station. A conductivity-temperature-depth (CTD) cast was also conducted to within 5 m of the bottom at each station.

Before they were sorted, ichthyoplankton samples were rinsed in fresh water with a 332-μm mesh sieve. The samples were then poured into a large Pyrex dish on a light box. All fish larvae and eggs were removed from the samples. Identifications were made to the lowest possible taxa by using a dissecting microscope and taxonomic information, primarily from Matarese et al. (1989) and Moser (1996). Zooplankton volume was determined by allowing the sample to settle in a graduated cylinder overnight. Zooplankton volumes and ichthyoplankton densities were standardized by adjusting these numbers by the volume of water filtered by the net.

Data analysis

Species diversity was calculated with the Shannon-Weaver index of diversity, H' (Shannon and Weaver, 1949; Krebs, 1989), where higher values denote greater species diversity. Species evenness was calculated with Simpson's index of evenness, λ, which ranges between 0 and 1, and where a value of 1 indicates that all taxa have the same density within a sample (Krebs, 1989).

To eliminate the effect of rare and uncommon taxa, data from each station were filtered to remove those taxa that occurred in less than 5% of the samples, thus leaving 12 egg taxa and 12 larval taxa for further analysis of the community structure. Agglomerative hierarchical two-way cluster analyses were conducted to identify taxa and sample assemblages. For this analysis, densities were averaged from both stations from a single collection date. Samples with no ichthyoplankton were excluded. Catch distributions were highly skewed and thus were $\log_{10}(catch/1000\ m^3+1)$ transformed to de-emphasize the few high catches. We constructed separate two-way clusters for eggs and larvae data with the Bray-Curtis distance measure and a flexible beta (β=–0.25) clustering algorithm, using PC-ORD software (vers. 5, MJM Software Design, Gleneden Beach, OR).

A nonparametric multiresponse permutation procedure (MRPP) (Kruskal, 1964; Mather, 1976) was used to test the hypothesis of no difference between two or more groups. Factors tested were station (buoy 1 and 2), year (1999, 2000, 2001, 2002, 2003, 2004), and season (downwelling and upwelling), before and after the spring transition date as defined by Logerwell et al. (2003). The date of the spring transition is the day when the ocean conditions switch from downwelling to upwelling, as identified by analyzing Bakun upwelling indices and sea level conditions along the Pacific Northwest (Logerwell et al., 2003). Identification of the primary taxa associated with each grouping was done by using an indicator species analysis (ISA). The ISA measures the fidelity of a taxon within a particular group in relation to its abundance in all groups and assigns an indicator value for that taxon per group. A statistical significance was then calculated by a Monte Carlo resampling method with 1000 runs. These tests were also run with PC-ORD V5 software. Relationships between environmental conditions and the ichthyoplankton community were investigated by using the BVSTEP procedure in the PRIMER software package (vers. 5, PRIMER-E Ltd, Lutton Ivybridge, UK). This analysis is analogous to forward stepwise multiple regression and compares nonparametrically paired species and environmental similarity matrices. Species similarity matrices were calculated by using Bray-Curtis similarities on fourth-root transformed data. Environmental matrices

were calculated by using the normalized Euclidean distance on fourth-root transformed data. Environmental variables in the analysis included sea surface temperature, salinity, chlorophyll *a* at 3 m, the settled volume of zooplankton, and Columbia River flow (measured by U.S. Geological Survey at Beaver, OR).

A paired *t*-test was used to determine if environmental conditions and zooplankton volumes at the two stations were significantly different. To account for interannual and seasonal variations in sampling, only those samples collected within a similar season were considered. All statistical tests were considered significant at the $\alpha < 0.05$ level.

Results

In total, 85 samples were collected during this study, comprising a total of 155,302 fish eggs and 3565 fish larvae, representing 34 taxa and 17 families. However, a majority (n=78) of these samples were taken from April to August and formed the primary data set that was analyzed (Table 2). The family Pleuronectidae had the greatest number of taxa (9), followed by the family Cottidae (6). The actual number of fish species collected was probably higher than the number we report because some eggs and larvae could be identified only to genus or family, namely eggs and and larvae of Osmeridae, Bathylagidae, *Citharichthys* spp., *Sebastes* spp., and *Sebastolobus* spp. Nine taxa were found only once during our sampling.

The most abundant fish eggs were those of northern anchovy, sanddab (*Citharichthys* spp.), blacksmelt (*Bathylagus* spp.), jack mackerel (*Trachurus symmetricus*), and Pleuronectidae, in descending order of abundance (Fig. 2A). The most abundant larvae, in descending order, were northern anchovy, English sole (*Parophrys vetulus*), sand sole (*Psettichthys melanostictus*), rex sole (*Glyptocephalus zachirus*), and Pacific tomcod (*Microgadus proximus*) (Fig. 2B). The unidentified Pleuronectidae eggs were of four species (butter sole [*Isopsetta isolepis*], English sole, starry flounder [*Platichthys stellatus*], and sand sole) which cannot be identified to species at early egg stages. Unusual egg occurrences included those of Pacific viperfish (*Chauliodus macouni*), opah (*Lampris guttatus*), and jack mackerel. These species are considered either offshore (viperfish and opah) or southern, warm-water spawners (jack mackerel). Jack mackerel eggs were observed in 1999–2002, but not in 2003 or 2004 (Fig. 2A). Peak jack mackerel spawning occurred in July 1999 and June 2000–2002, although some eggs were also found from May to September during most years.

Northern anchovy accounted for 76% of all eggs collected, and were found from May through July (Fig. 3). Overall, northern anchovy were present in 53 of the total 85 samples collected. Peak northern anchovy egg abundance varied interannually, occurring in June of 1999, 2001, and 2002; July of 2000; and in May of 2003. The highest annual density of northern anchovy eggs was observed in 2003, when eggs were almost four times as abundant as those observed in 2002 (Fig. 3). Northern anchovy eggs dominated the catch in all years except 2001, when blacksmelt and sanddab contributed about equally to the catch (Fig. 2).

Northern anchovy larvae were the most abundant fish larvae, and represented 68% of all larvae collected. In 1999, 2001, and 2002, peak numbers of these larvae occurred in June, whereas in 2000, northern anchovy larval numbers peaked in July. In 2003 and 2004, northern anchovy larval numbers peaked in May and represented the majority of the larval fish catch (Fig. 2B).

Nine samples contained only one egg or larval fish species (and therefore a diversity and evenness of zero), and three samples contained no ichthyoplankton. Overall sample diversity ranged

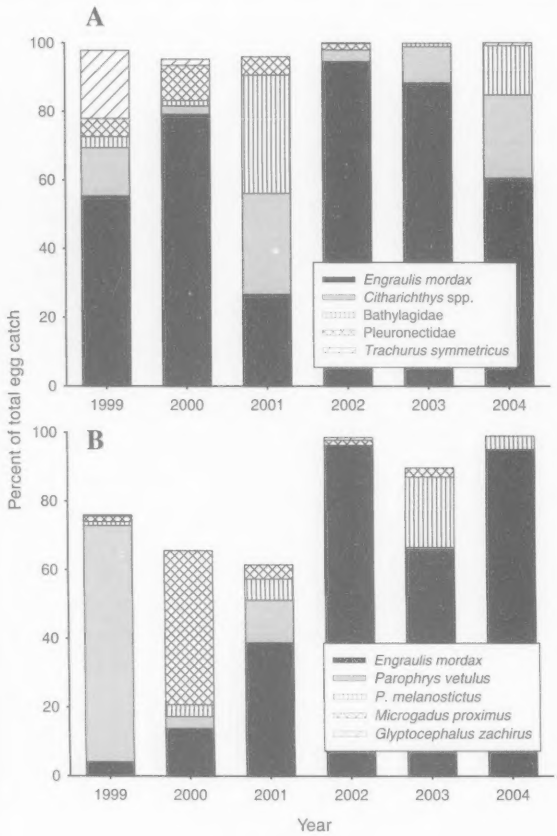

Figure 2

The top five egg (**A**) and larval (**B**) taxa collected at two stations off the mouth of the Columbia River, Oregon, as a percentage of total catch for each year from 1999 through 2004.

Table 2

Taxonomic and stage composition of ichthyoplankton collected during survey cruises conducted by the National Oceanic and Atmospheric Administration, National Marine Fisheries Service approximately every two weeks from late April to August 1999–2004 from two stations located off the Columbia River, Oregon. Occurrence is the number of samples in which the life stage of a taxon was found.

Species	Common name	Occurrence (n=78)	Density (no. /1000 m^3)	Number captured	Stage collected
Clupeidae					
Sardinops sagax	Pacific sardine	4	0.00899	86	Eggs
Engraulidae					
Engraulis mordax	Northern anchovy	52	7.09502	118,089	Eggs
		23	0.18015	2435	Larvae
Bathylagidae	Blacksmelts	18	0.81678	11,027	Eggs
Chauliodontidae					
Chauliodus macouni	Pacific viperfish	2	0.00012	2	Eggs
Gadidae					
Microgadus proximus	Pacific tomcod	13	0.00278	32	Larvae
Lampridae					
Lampris guttatus	Opah	1	0.00011	1	Eggs
Gasterosteidae	Stickleback	1	0.0001	1	Larvae
Scorpaenidae					
Sebastes spp.	Rockfish	2	0.00021	2	Larvae
Sebastolobus spp.	Thornyheads	5	0.0004	6	Eggs
Hexagrammidae					
Ophiodon elongatus	Lingcod	2	0.00186	2	Larvae
Cottidae					
Artedius spp.[1]		1	0.00007	1	Larvae
Artedius harringtoni	Scalyhead sculpin	2	0.00031	5	Larvae
Clinocottus acuticeps	Sharpnose sculpin	1	0.00012	1	Larvae
Leptocottus armatus	Staghorn sculpin	1	0.00026	2	Larvae
Ruscarius meanyi	Puget Sound sculpin	1	0.00008	1	Larvae
Liparidae					
Liparis spp.	Unid. snailfish	2	0.00018	3	Larvae
Liparis fucensis	Slipskin snailfish	1	0.00008	1	Larvae
Liparis pulchellus	Showy snailfish	2	0.00025	3	Larvae
Carangidae					
Trachurus symmetricus	Jack mackerel	15	0.19986	2178	Eggs
Bathymasteridae					
Ronquilis jordani	Northern ronquil	1	0.00095	12	Larvae
Pholidae					
Pholis spp.	Unid. gunnel	1	0.00004	1	Larvae
Icosteidae					
Icosteus aenigmaticus	Ragfish	2	0.00034	4	Eggs
Ammodytidae					
Ammodytes hexapterus	Pacific sandlance	2	0.00187	2	Larvae
Paralichthyidae					
Citharichthys spp.	Sanddab	50	1.66568	22,186	Eggs
		1	0.00008	1	Larvae
Pleuronectidae	Unid. flounder	20	0.04348	673	Eggs
		2	0.00029	3	Larvae
Glyptocephalus zachirus	Rex sole	6	0.00667	34	Eggs
		3	0.00546	5	Larvae
Isopsetta isolepis	Butter sole	6	0.00072	9	Larvae
Lepidopsetta bilineata	Southern rock sole	1	0.00029	4	Larvae

continued

Table 2 (continued)

Species	Common name	Occurrence (n=78)	Density (no. /1000 m^3)	Number captured	Stage collected
Pleuronectidae (cont.)					
Lyopsetta exilis	Slender sole	4	0.00082	9	Eggs
		2	0.00016	2	Larvae
Microstomus pacificus	Dover sole	8	0.00566	65	Eggs
		4	0.00123	24	Larvae
Parophrys vetulus	English sole	6	0.00101	11	Larvae
Platichthys stellatus	Starry flounder	1	0.00016	2	Larvae
Pleuronichthys decurrens	Curlfin sole	4	0.00504	5	Eggs
		1	0.00015	2	Larvae
Psetticthys melanostictus	Sand sole	7	0.00920	112	Eggs
		17	0.01202	164	Larvae

[1] Either *Artedius corallinus* or *A. notospilotus*.

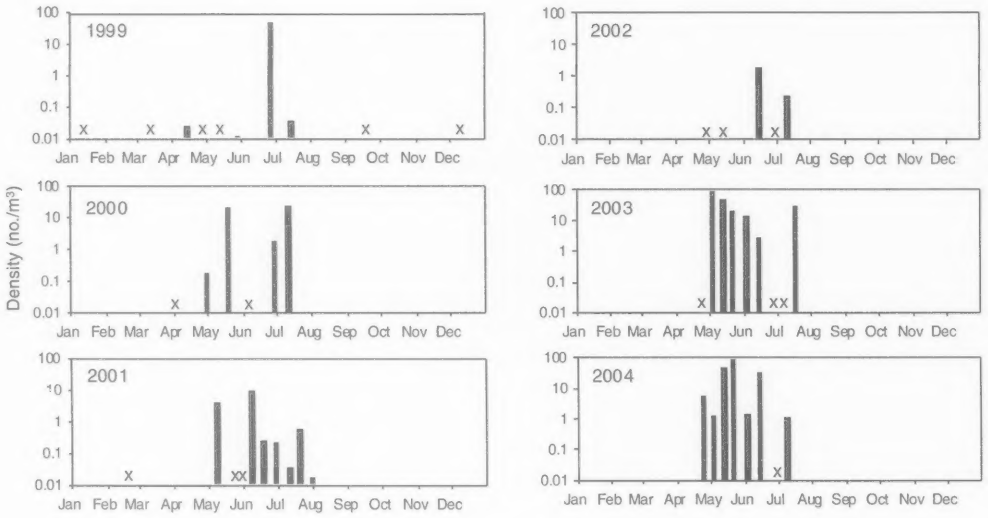

Figure 3

Densities of northern anchovy (*Engraulis mordax*) eggs sampled off the mouth of the Columbia River, Oregon, in relation to sampling date for 1999 through 2004 (note log scale on y-axis). An X represents dates when sampling occurred but no northern anchovy eggs were collected.

from 0 to 1.7 and evenness from 0 to 1. Over all years, diversity was highest in April for eggs at both stations and for larvae at buoy 1, whereas at buoy 2, peak diversity for larvae occurred in March. Lowest diversities occurred in late fall and especially in December. Evenness was highest in April and lowest in December.

More than half of all species included in the egg-stage cluster analysis were flatfish from the families Pleuronectidae and Paralichthyidae (Fig. 4) . The most closely grouped species were the northern anchovy and sanddab. Other taxa with a similar abundance pattern included the blacksmelt (Bathylagidae) and Pleuronectidae. Three sampling groups emerged in the egg cluster analysis (Fig. 4). The first group or cluster (A) consisted mainly of samples collected in winter–spring (December–early May), before the spring transition, and was dominated by rex sole and other flatfishes. Cluster B was composed of mainly late spring (April–June) samples and was dominated by jack mackerel and sanddab eggs. In contrast, Cluster C was predomi-

nately a spring–summer (May–July) group dominated by northern anchovy, sanddab, and blacksmelt (Fig. 4).

Among the larvae, Pacific sandlance (*Ammodytes hexapterus*), rex sole, and curlfin sole (*Pleuronichthys decurrens)* were tightly clustered, as were northern anchovy and thornyheads (*Sebastolobus* spp.) (Fig. 5). The remaining larger clusters consisted of mainly flatfishes and Pacific tomcod. Sampling clusters were less defined for larval fish than for eggs (Fig. 5). Cluster A consisted of samples collected from multiple years and months, and most were collected before the spring transition. No species dominated these collections. In contrast, Cluster B was dominated by Pacific tomcod and Dover sole (*Microstomus pacificus*). Cluster C occurred entirely during May and June and was distinguished by the high abundance of northern anchovy (Fig. 5).

There were very limited differences in the biological or physical oceanographic conditions between the two sampling stations. Only salinity was significantly different between the two stations (t-test, $P<0.05$). Temperature, chlorophyll a, zooplankton densities, and densities of the five most abundant ichthyoplankton species were not significantly different between stations. However, there were significant annual differences in temperatures (ANOVA, $P<0.05$), but not salinities or chlorophyll a at these stations during the study period (Table 3). There were also large annual differences in the date of the spring transition (Table 3). The BVSTEP analysis revealed that temperature explained most of the interannual variation in the ichthyoplankton community (Spearman $\rho=0.345$), (ρ ranges from –1 to 1, where values farthest from zero indicated a stronger correlation) followed by salinity ($\rho=0.194$) and Columbia River flow ($\rho=0.145$). Chlorophyll a and overall ichthyoplankton densities did not provide any explanatory power and did not relate to variations in ichthyoplankton species composition.

The egg and larval fish data showed very few differences when these data were grouped and compared by year, season, and station. MRPP analysis revealed few significant differences between groups (Table 4). The taxonomic composition of the dominant egg or

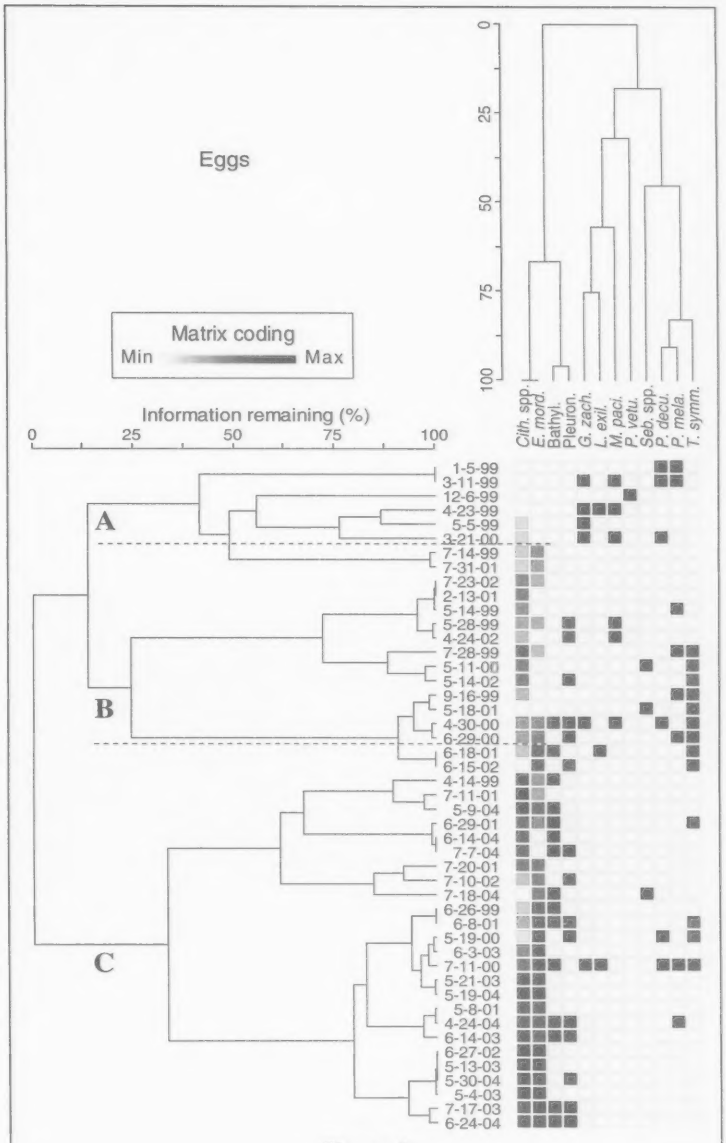

Figure 4

Two-way cluster analysis of egg densities by sampling date. The taxa clusters are oriented vertically at the top of the figure and sampling date clusters are oriented horizontally at the left side of the figure. The dashed lines delineate sampling dates that contained similar assemblages of eggs. The color of the boxes denotes relative abundance of each taxon during each sampling period as shown in the matrix coding legend. The taxa names are abbreviated as: *Cith.* spp. (*Citharichthys* spp.), *E. mord.* (*Engraulis mordax*), Bathyl (Bathylagidae), Pleuron. (*Pleuronectidae*), *G. zach.* (*Glyptocephalus zachirus*), *L. exil.* (*Lyopsetta exilis*), *M. paci.* (*Microstomus pacificus*), P. vetu. (*Parophrys vetulus*), *Seb.* spp. (*Sebastolobus* spp.), *P. decu.* (*Pleuronichthys decurrens*), *P. mela.* (*Psetticthys melanostictus*), and *T. symm.* (*Trachurus symmetricus*). "Information remaining" is a scaled measure of the amount of information that is left after stations are grouped.

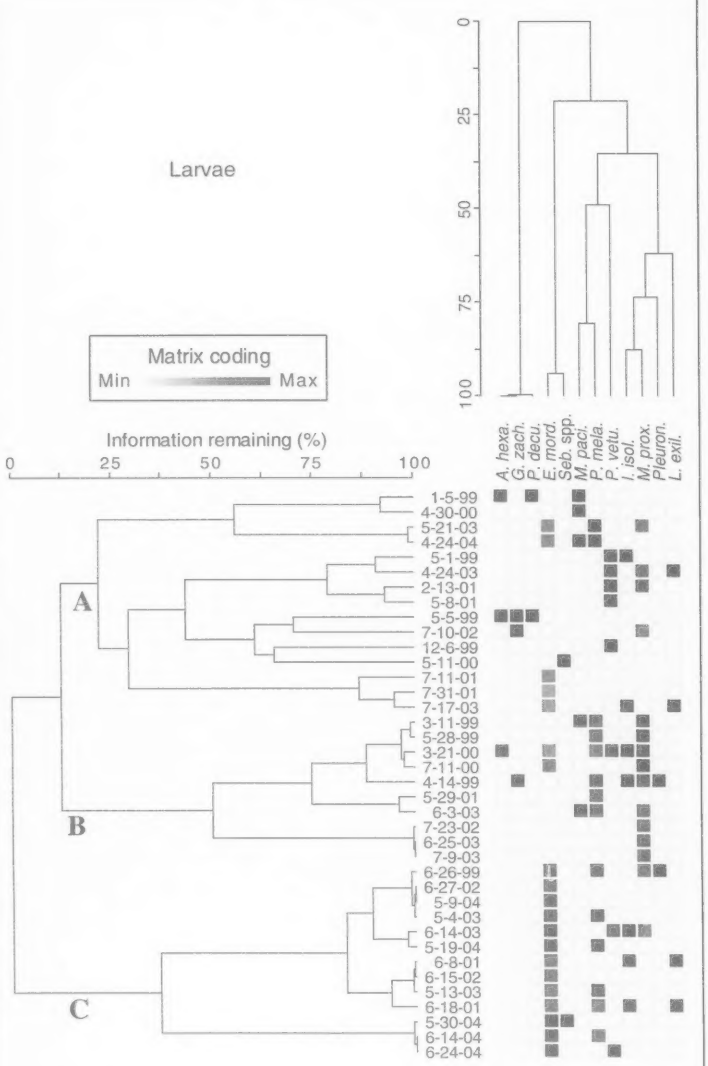

Figure 5

Two-way cluster analysis of larval densities by sampling date. The taxa clusters are oriented vertically at the top of the figure and sampling dates are oriented horizontally at the left side of the figure. The dashed lines delineate sampling dates that contained similar assemblages of larvae. The color of the boxes denotes relative abundance of each taxon during each sampling period as shown in the matrix coding legend. The taxa names are abbreviated as: *A. hexa.* (*Ammodytes hexapterus*), *G. zach.* (*Glyptocephalus zachirus*), *P. decu.* (*Pleuronichthys decurrens*), *E. mord.* (*Engraulis mordax*), *Seb.* spp. (*Sebastes* spp.), *M. paci.* (*Microstomus pacificus*), *P. mela.* (*Psetticthys melanostictus*), *P. vetu.* (*Parophrys vetulus*), *I. isol.* (*Isopsetta isolepis*), *M. prox.* (*Microgadus proximus*), Pleuron. (Pleuronectidae), and *L. exil.* (*Lyopsetta exilis*). "Information remaining" is a scaled measure of the amount of information that is left after stations are grouped."

larval taxa did not differ between the two stations, and no indicator species were identified for either station. Year of sampling was a significant factor for eggs; 2003 and 2004 showed a different composition from most other years. However, eggs of only 3 of the 12 species (curlfin sole and jack mackerel for 2000; northern anchovy for 2003) were determined to be useful indicator species. Season was not a significant factor overall for eggs, or for the two taxa considered indicators of downwelling and upwelling conditions. For larvae, year was a marginally insignificant factor overall, although there were pairs of years that were significantly different. Season was a significant factor for larvae: Dover sole and English sole larvae were indicative of winter downwelling, and northern anchovy were indicative of summer upwelling seasons. Station was not a significant factor for eggs or larvae.

Discussion

The timing of upwelling (spring transition), and its associated ocean temperatures, appears to be very important in determining the structure of the ichthyofaunal community off the Columbia River and probably within the California Current. Upwelling is what brings nitrates into the Columbia River plume (Lohan and Bruland, 2006) and the California Current and ultimately determines primary and probably secondary production (Hickey and Banas, 2003). As such, it is not surprising that fishes within this region have evolved life histories adapted to its presence (Bowen and Grant, 1997; Ware and Thomson, 1991). However, although upwelling drives the nutrient enrichment processes it also can disrupt the abilities of larval coastal fishes to successfully recruit if they are unable to take advantage of transport and retention processes (Bakun, 1996).

Several factors account for the high abundance of northern anchovy eggs and larvae captured during our study. The Columbia River plume is an important spawning habitat for northern anchovy (Richardson, 1981; Emmett et al., 1997) and most samples were collected during the typical northern anchovy spawning season (May–August). Fur-

Table 3

Average annual 3-m salinity, temperature, and chlorophyll *a* values at two stations off the Columbia River, Oregon. Also shown is average spring (April–June) Columbia River flows and day of the spring transition. The day of the spring transition (when upwelling begins) was identified with the methods of Loggerwell et al., 2003. Only temperature and Columbia River flows were found to have significant annual differences (ANOVA, $P<0.05$).

Year	Salinity	Temperature (°C)	Chlorophyll *a* (μg/L)	Columbia River flows (m^3/s)	Day of spring transition
1999	28.53	11.51	4.81	9999	1 Apr
2000	29.92	12.61	2.34	8132	12 Mar
2001	29.19	13.28	2.66	4506	2 Mar
2002	27.93	12.61	6.12	8640	21 Mar
2003	26.68	12.93	5.61	8316	22 Apr
2004	27.50	14.09	5.07	6777	19 Apr

Table 4

Results of the multi-response permutation procedure (MRPP) and indicator species analysis (ISA) for annual, seasonal (before and after the spring transition [Logerwell et al., 2003]), and station differences in composition of the dominant egg and larval ichthyoplankton taxa. Shown are the MRPP *A*-statistic, overall significance value, and significant pair-wise comparisons that emerged. The significant indicator species are listed with the year or season with which each species is associated in parentheses.

Stage	Variable	MRPP *A*-statistic	*P* value	Significant differences between levels	Significant indicator species
Eggs	Year	0.1253	<0.001	2003 ≠ all years but 2004 2004 ≠ all years but 2002, 2003	*Pleuronichthys decurrens*, *Trachurus symmetricus* (2000), *Engraulis mordax* (2003)
	Season	0.0231	0.087	None	*Psetticthys melanostictus*, *Microstomus pacificus* (downwelling), *E. mordax*, *Citharichthys* spp. (upwelling)
	Station	0.0019	0.631	None	None
Larvae	Year	0.1027	0.068	2003 ≠ 1999 and 2004 2002 ≠ 2004	*Parophrys vetulus*, *M. pacificus* (downwelling), *E. mordax* (2004)
	Season	0.0787	0.018	downwelling ≠ upwelling	*E. mordax* (upwelling)
	Station	0.0003	0.412	None	None

thermore, northern anchovy eggs are positively buoyant and appear to concentrate in plume fronts (Morgan et al., 2005), which often occurred in our sampling region. The observed annual increase in northern anchovy egg densities appear to be strongly linked to the increase in the abundance of adult northern anchovy population in the study area (Emmett et al., 2006). Anchovy egg densities also may have been influenced by Columbia River spring flows. For example, densities of northern anchovy egg and larvae were lower in 2001, a drought year during which Columbia River flows were very low and the plume area was relatively small (Brodeur et al., 2005). However, numbers of northern anchovy eggs were also down in 2002, which was not a drought year, but was the year that the Pacific Decadal Oscillation (PDO) changed from negative to positive (Goericke et al., 2005).

Richardson (1981) found that the peak of northern anchovy spawning in 1975 and 1976 occurred in July, whereas we found that the peak of spawning occurred in May during five of the six study years. However, this difference could have been due to Richardson (1981) not sampling in May and June. Brodeur et al. (1985) found northern anchovy spawning during April 1983, but 1983 was a strong El Niño year and therefore there were unusually warm ocean conditions. Jones et al. (1990) found northern anchovy eggs and larvae in the Columbia River estuary from April to September 1980,

with a peak in June. We are not certain whether the timing of northern anchovy spawning has shifted to earlier months, whether this early spawning was missed by previous surveys, or whether we have two different age-groups spawning at different times. Nevertheless, our results indicate that timing of northern anchovy egg surveys is an important parameter to consider during surveys to estimate spawning biomass (Emmett et al., 1997) and feeding success relative to plankton production cycles (Bollens et al., 1992).

Jack mackerel, which generally spawn off southern California (MacCall and Stauffer, 1983), are not commonly collected as juveniles very far north in the California Current. Because jack mackerel eggs hatch in 4.3 days at 12°C (Farris, 1961) and because we observed jack mackerel larvae, it appeared that jack mackerel spawned in the general vicinity of our study area. Jack mackerel spawning has been reported off Washington State and southern Oregon (Ahlstrom, 1956), but not for many years. Ahlstrom (1956) noted that there is a northward progression of the spawning season in areas north of Point Conception, California. During our study period, it was possible that adult jack mackerel moved north and into cooler waters inshore to feed and spawn. The recent capture of age-0 jack mackerel during pelagic fish surveys off Oregon and Washington (Brodeur et al., 2006) indicates that jack mackerel have recently spawned and recruited successfully off the Pacific Northwest coast.

The occurrence of rare taxa, such as opah, can be attributed to the long incubation time (up to 3 weeks) exhibited by most lampriform fish eggs (Olney, 1984). In this time, an egg spawned in the open ocean, the normal habitat of this species, could drift inshore to our sampling area. Similarly, Pacific viperfish eggs, released in the open ocean, can drift inshore. Little is known about their egg incubation period.

The occurrence of several offshore ichthyoplankton species in our study area may be related to the local topography. The Astoria Canyon lies directly seaward from the mouth of the Columbia River. During the upwelling season, the canyon causes currents to flow landward (Hickey, 1997), thus carrying normally offshore organisms closer to shore. Bosley et al. (2004) concluded that currents over the Astoria Canyon concentrate oceanic organisms and transport them shoreward and these actions may explain the occurrences of opah, Pacific viperfish, and jack mackerel eggs, and other offshore species (Richardson and Pearcy, 1977) in our coastal sampling.

Egg and larval composition at the two sampling stations did not differ significantly, probably because they were relatively close and thus had similar environmental conditions. Out of a total of five physical variables tested, only salinity was found to be significantly different between the two stations. Surface salinity is highly variable in this region because of the proximity of the Columbia River plume. While the plankton tow depths at each station were the same (40 m), we would have missed eggs and or larvae occurring below 40 m at the station farther offshore (buoy 1), which was in substantially deeper water. Boehlert et al. (1985) and Auth and Brodeur (2006) found fish larvae at depths greater than 40 m off the coast of Oregon, although the majority of fish larvae were in surface waters.

The cluster dendrograms were dominated by flatfish species because of the high number of pleuronectid species found along the Oregon coast, as well as the tendency of most of these species to have pelagic eggs. Overall patterns in the egg and larval clusters changed seasonally, and many of the samples collected during the downwelling or upwelling seasons were clustered together. MRPP analysis verified that season was an significant contributor to these cluster groups. The larval indicator species for downwelling conditions were English sole and Dover sole, which are typically winter spawners (although some spawning may occur all year). The indicator species for the upwelling season was northern anchovy, which spawns primarily in the spring and summer. In general, benthic and nearshore species spawn in winter, when larvae are less likely to be transported offshore. In 2003, the PDO shifted from a cool (negative) to a warm (positive) phase. This change resulted in southern or offshore taxa becoming more abundant in 2003–04. However, ichthyoplankton taxa in 2003 and 2004 were also significantly different mainly because of substantial increases in eggs and larvae of northern anchovy, a taxon that is not southern affiliated. The increase in northern anchovy eggs and larvae appeared linked to an increase in the abundance of the adult population.

Large-scale climate variability (as observed during our study period) can cause large changes in fish populations (Cushing, 1982; Beamish, 1993; Francis et al., 1998; Chavez et al., 2003). Pearcy (2002) and Brodeur et al. (2003, 2006) found that abnormal ocean conditions alter the ichthyofauna in the California Current region. Changing climate conditions can alter currents that advect fish eggs and larvae to or away from nursery areas, thus affecting recruitment. Altered currents may also lower the density and change the species composition of planktonic food organisms (Peterson and Schwing, 2003) and thus inhibit larval fish from finding adequate prey resources. In late 2002, the PDO (Mantua et al., 1997) became positive (with warm conditions) after four years of being negative (with cold conditions) (Goericke et al., 2005) and caused warmer ocean temperatures, increased diversity of warm-water copepods, and decreased cold-water copepods in the California Current (Hooff and Peterson, 2006). Changes in ocean temperatures can also cause shifts in the locations or timing of spawning and affect developmental durations through the early life stages of fishes (Sabatés et al., 2006; Phillips et al., 2007). Faster development through the egg and yolk-sac stages may help to minimize the time eggs are vulnerable to invertebrate predation (Bailey, 1981). In the California Current region, upwelling is the dominant feature that influences primary production, and therefore any climate-induced change that affects upwelling or Califor-

nia Current circulation patterns will alter zooplankton prey that larval fish feed on.

Climate-induced change in upwelling patterns, sea surface temperatures, and perhaps Columbia River flows will have large effects on the larval fish within our study area and in the broader California Current region. We examined interannual variation in the abundance of fish eggs and larvae in the Columbia River plume and have shown that the ichthyoplankton community changes in relation to the dynamic oceanographic processes of this region. However, research over a greater area and on a longer time scale is needed to determine the effects of these processes on the ichthyoplankton community, as well as to determine how recruitment of marine fish is being affected by regional and larger-scale variability in the marine environment.

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Abstract—The migratory population of striped bass (*Morone saxatilis*) (>400 mm total length [TL]) spends winter in the Atlantic Ocean off the Virginia and North Carolina coasts of the United States. Information on trophic dynamics for these large adults during winter is limited. Feeding habits and prey were described from stomach contents of 1154 striped bass ranging from 373 to 1250 mm TL, collected from trawls during winters of 1994–96, 2000, and 2002–03, and from the recreational fishery during 2005–07. Nineteen prey species were present in the diet. Overall, Atlantic menhaden (*Brevoortia tyrannus*) and bay anchovy (*Anchoa mitchilli*) dominated the diet by biomass (67.9%) and numerically (68.6%). The percent biomass of Atlantic menhaden consumed increased from 50.3% during 1994–2003 to 87.0% during 2005–07. Demersal fish species such as Atlantic croaker (*Micropogonias undulatus*) and spot (*Leiostomus xanthurus*) represented <15% of the diet biomass, whereas alosines (*Alosa* spp.) were rarely observed. Invertebrates were least important, contributing <1.0% by biomass and numerically. Striped bass are capable of feeding on a wide range of prey sizes (2% to 43% of their total length). This study outlines the importance of clupeoid fishes to striped bass winter production and also shows that predation may be exerting pressure on one of their dominant prey, the Atlantic menhaden.

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Interactions between adult migratory striped bass (*Morone saxatilis*) and their prey during winter off the Virginia and North Carolina Atlantic coast from 1994 through 2007

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Striped bass (*Morone saxatilis*) has a well documented history along the U.S. east coast, from its dramatic population declines during the 1980s to its subsequent recovery by the early 1990s (Field, 1997; Richards and Rago, 1999). Successful management efforts have resulted in a greater than tenfold increase in striped bass abundance between the 1980s and 1990s and a subsequent increase in population-level prey consumption (Hartman, 2003), and therefore a concern for coastal populations of prey species (Hartman, 2003; Overton, 2003; Uphoff, 2003). Under current management regimes, it may be difficult to maintain high population levels of striped bass and their prey (Hartman, 2003; Uphoff, 2003).

Typically, striped bass along the U.S. east coast spend their first years maturing in their natal estuaries and then emigrate to the Atlantic Ocean. Most striped bass along the Atlantic coast migrate northward during spring and summer to waters off the northeast coast of the U.S. During fall and winter, they return south to overwinter off the coasts of Virginia and North Carolina (Boreman and Lewis, 1997). These migratory fish are generally large (>500 total length [TL] mm) and feed prodigiously during their migrations. Theoretically, these large piscivores are capable of structuring prey fish populations through predation and prey selection (Bax, 1998; Harvey et al., 2003), and in turn they can potentially influence the recruitment success of prey species. Predators such as striped bass are capable of consuming prey that are a wide range of sizes (Hartman, 2000); therefore to understand trophic relationships it is important to examine their dietary habits. Multispecies fisheries and ecosystem management approaches require dietary information for upper-level predators such as striped bass (Latour et al., 2003).

The literature on diets and feeding habits of striped bass (see Walter et al., 2003) is voluminous. However, information on feeding habits during

their oceanic migrations in winter is limited. Walter et al. (2003) identified the paucity of information on the foraging habits of striped bass along the Atlantic coast during their winter residency as one of the major gaps in the life history of this species. This paucity of information about feeding habits during winter is especially acute, given the importance of predator-prey interactions and their relation to the population base in the area. The objective of this paper was to gather and synthesize detailed information on annual feeding habits of striped bass during winter off the coasts of Virginia and North Carolina from 1994 through 2007. Therefore, we determined the important prey types and the prey-size spectrum of striped bass during winter.

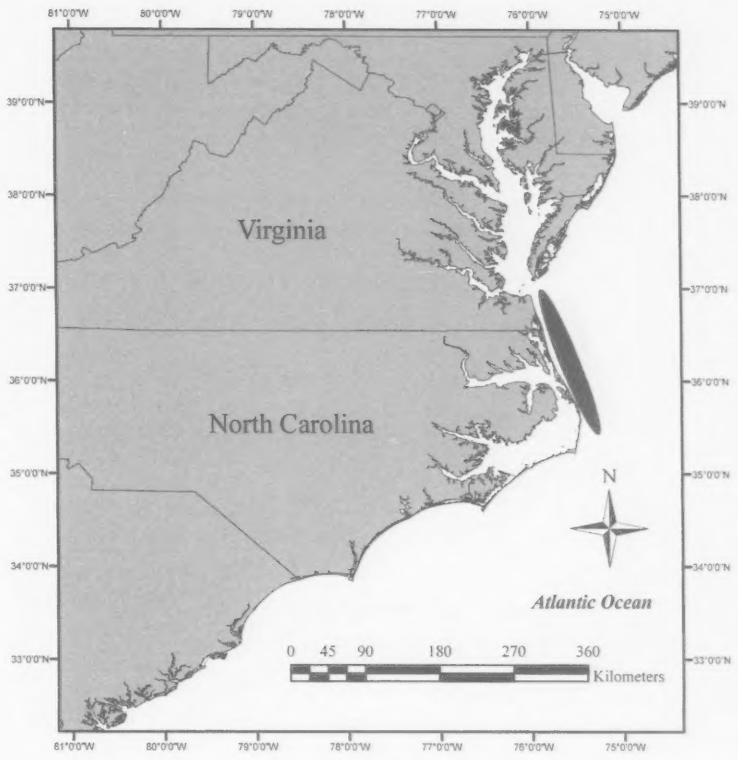

Figure 1

Map of U.S. east coast and the general sampling area (shaded oval shape) for striped bass (*Morone saxatilis*) collected in trawl and recreational catch samples from 1994 through 2007.

Materials and methods

Striped bass were collected by two methods: trawls and dockside sampling of the catch of recreational fishermen. Beginning in 1988, a number of fisheries management agencies (National Marine Fisheries Service, United States Fish and Wildlife Service, North Carolina Department of Marine Fisheries, Maryland Department of Natural Resources, and Virginia Marine Resource Commission) organized a trawl survey for striped bass from federal research vessels during winter off the coast of Virginia and North Carolina (Fig. 1). The primary objective of the survey was to tag and release striped bass to assess annual mortality of the coastal migratory stock. The trawl specification and trawl duration varied over the years. Generally, trawl sampling occurred around the clock during mid-January. Once the trawl was recovered, most striped bass were tagged and released; however, dead or moribund fish not selected for tagging were sacrificed and processed for aging or food habit analyses. Few fish (n<19) were examined for stomach contents from 1997–99, 2001, and 2004–07; therefore data from these years were excluded from our analyses.

From December to March 2004–07, striped bass were collected weekly at the Oregon Inlet Fishing Center (OIFC) in Manteo, North Carolina. Fish were sampled from recreational fishermen who brought their daily catches to the fish cleaning station at the OIFC. We randomly selected fish once they were cleaned (generally filleted), measured the carcasses for total length (±1.0 mm), and identified the sex of each individual. Stomachs were removed and frozen for later analysis. Additional samples were obtained from Virginia Beach Fishing Center, Virginia Beach, Virginia and processed in the same manner as that used to process fish from the OIFC. All stomach samples presumably came from fish captured in the Atlantic Ocean 0–4.8 km from the shoreline because no fishing for striped bass is permitted in the U.S. Exclusive Economic Zone (EEZ), beyond 4.8 km (3 miles) from shore.

In the laboratory, stomach contents were thawed and all prey items removed, sorted, identified (to the lowest taxon possible, usually to species for fish and decapod crustaceans and family for other invertebrates), enumerated, weighed to the nearest 0.1 g, and measured to the nearest mm (standard, carapace, or total length). The percentage of prey by number and percent composition by weight (wet weight-biomass) were determined. A quantitative assessment of number and weight of each prey item was used, as well as the respective percentage values for each (Markle and Grant, 1970; Macdonald and Green, 1983). Percent weight is a measure of the nutritional value of the prey (Macdonald and Green, 1983) and is calculated as the total weight of each prey category divided by the total weight of all prey categories. Frequency distributions of prey

total length to predator total length (prey-to-predator ratios; PPR) were examined. We used one-way analysis of variance (ANOVA) to test for differences in mean length (Log_{10} transformed) between the fish from the trawl and recreational samples ($P \leq 0.05$). We also fitted a least squares linear regression of prey total length and striped bass total length.

Results

We collected 263 stomachs from striped bass in the trawl samples from 1994 through 2003 (Table 1); specimens ranged from 373 to 955 mm TL (mean=662.2 ±129.1 standard deviation (SD); Fig. 2). The percentage of stomachs that contained food ranged from 73.5% to 100% (mean=84.6%). From the recreational samples (2005–07), 891 fish were examined (Table 1). The striped bass size ranged from 509 to 1250 mm TL (mean=918.9 ±93.8 SD; Fig. 2). The size of fish collected from the recreational catch were significantly larger (ANOVA, P=<0.0001; df =1) than those collected by trawl.

The percentage of stomachs containing food items was more variable for striped bass caught by recreational anglers (23.6–80.7%), than for the fish caught by trawl (73.5–100.0%). In 2005, 23.6% of the stomachs contained food; increased to 24.4% in 2006, and to 80.7% by 2007. Collectively, 19 fish and invertebrate species constituted the diet of striped bass (Table 2), and fish predominated.

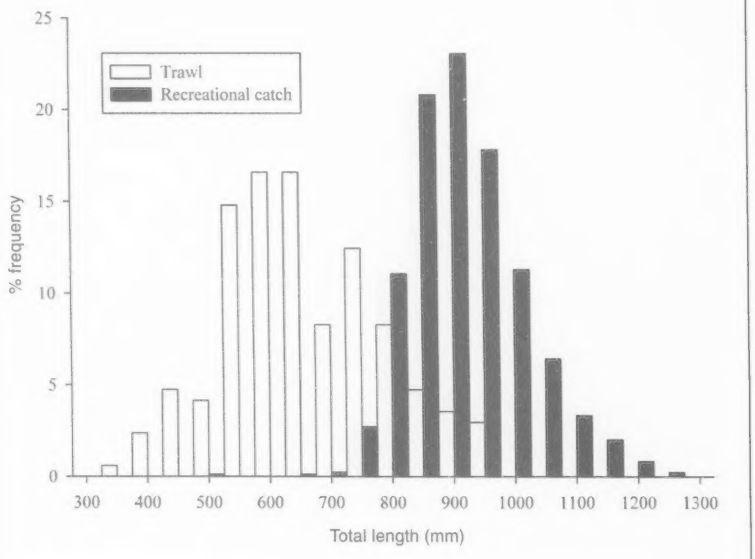

Figure 2

Length-frequency histogram for striped bass (*Morone saxatilis*) collected during the winter off the coasts of Virginia and North Carolina during 1994–2007.

Feeding habits (trawl samples 1994–2003)

Atlantic menhaden and bay anchovy were the most abundant species present in striped bass stomachs in all years sampled; they also dominated the diet in biomass and numerically. Atlantic menhaden accounted 9.5% of the diet numerically and 50.3% by biomass (Fig. 3). The biomass of Atlantic menhaden was constant (40%) from 1994 to 2000; this contribution nearly doubled to 73.8% in 2002 and 72.4% by 2003. Atlantic menhaden showed no consistent pattern numerically and was generally <15%.

Concurrent with the increase in the biomass of Atlantic menhaden consumed was a decline in the percent biomass of bay anchovy found in the diet of striped bass. Bay anchovy accounted for 16.5% of the biomass to 29.9% of the diet numerically throughout the study period (Fig. 3). Between 1994 to 2000 mean percent biomass for bay anchovy was 43.4% and they represented 71.3% of the diet numerically. However, by 2002, the percent biomass declined to 16.7% and represented <6.6% in 2003. Bay anchovy dominated the diet by number, representing 94.5% in 1995 and remained >80% from 1996 through 2002 (Fig. 3).

Sciaenids and alosine species were minor contributors to the diet of striped bass. Weakfish (*Cynoscion regalis*) was absent from the diet before 2002 and represented <0.5% of the diet during the study (Table 2). From 1994 to 2003, Atlantic croaker (*Micropogonias undulatus*) was absent, except in 1995 and 2003 when it represented 10.1% and 14.7% of the diet biomass, respectively. Alosines (American shad [*Alosa sapidissima*]; blueback herring [*A. aestivalis*]; and hickory shad [*A. mediocris*]) were a minor part of the diet of striped bass, and they occurred only during 1994 and 1996. American shad were found once in 1996 (4.5% biomass, 6.8% numerically). Blueback herring were present in 1994 and 1996 and represented 3.1% and 8.1% of the diet biomass, respectively. Invertebrates were a minor portion of the diet of large striped bass generally contributing <1.0% to the diet (Table 2).

Feeding habits (determined from recreational catch samples 2005–07)

In the recreational catches, Atlantic menhaden and bay anchovy dominated striped bass diet both by biomass (88.9%) and numerically (93.6%). Biomass of Atlantic menhaden remained consistent

Table 1

Mean size (total length [TL] mm ±standard deviation [SD]), size range, and number of striped bass (*Morone saxatilis*) with food in their stomachs collected off the coasts of North Carolina and Virginia from trawl and hook-and-line samples during 1994–2007.

Collection year and gear type	Mean TL (mm, ±SD)	Size range (TL mm)	Number of fish examined (% with food in the stomach)
1994, trawl	613.1 (72.3)	425–765	73 (99)
1995, trawl	639.7 (57.0)	525–718	19 (100)
1996, trawl	805.9 (75.6)	666–955	34 (74)
2000, trawl	561.3 (90.5)	465–770	50 (84)
2002, trawl	616.2 (180.0)	373–941	60 (77)
2003, trawl	836.6 (80.6)	745–953	19 (84)
2005, hook-and-line	881.2 (94.5)	509–1150	253 (23)
2006, hook-and-line	914.6 (87.9)	720–1200	450 (28)
2007, hook-and-line	994.2 (99.0)	760–1250	140 (81)

Table 2

Diet summary of prey contributions (%B=biomass, %N=number) and mean prey size (total length [TL] ±standard deviation [SD]) of striped bass (*Morone saxatilis*) during the winter off the coast of North Carolina from 1994 through 2007. Asterisk represents only one prey item.

		Year									
		1994		1995		1996		2000		2002	
Fish	Scientific name	%B	%N	%B	%N	%B	%N	%B	%N	%B	%N
American eel	*Anguilla rostrata*										
American shad	*Alosa sapidissima*					4.5	6.8				
Atlantic croaker	*Micropogonias undulatus*			10.1	0.9						
Atlantic herring	*Clupea harengus*									4.1	0.4
Atlantic menhaden	*Brevoortia tyrannus*	38.8	10.6	29.9	4.5	30.5	2.2	31.8	13.7	73.8	7.3
Bay anchovy	*Anchoa mitchilli*	34.3	55.2	60.1	94.5	40.4	85.2	58.9	85.8	16.7	79.4
Blueback herring	*Alosa aestivalis*	3.1	6.1			8.1	0.5				
Butterfish	*Peprilus triacanthus*										
Hickory shad	*Alosa mediocris*										
Round herring	*Etrumeus teres*										
Sciaenid sp.		14.1	13.7								
Silver perch	*Bairdiella chrysoura*									1	0.4
Spot	*Leiostomus xanthurus*										
Tonguefishes	*Symphurus* sp.										
Unknown clupeid		1.6	2.1			16.2	4.8				
Fish remains		1.5	2.7			0.3	0.6	9.3	0.5	0.3	0.7
Weakfish	*Cynoscion regalis*									1.4	0.2
Menticirrhus spp.										0.3	0.2
Invertebrates											
Gastropod shell	Gastropod									2.4	11.4
Decapods	Decapoda	2.1	2.2								
Sand shrimp	*Crangon septimspinosa*	2.8	4.7								
Mud crab	*Panopeus herbstii*										
Longfin squid	*Loligo pealeii*										

continued

Table 2 (continued)

Fish	Scientific name	Year										Mean TL mm (±SD)
		2003		2005		2006		2007		Overall		
		%B	%N	%B	%N	%B	%N	%B	%N	%B	%N	
American eel	*Anguilla rostrata*			0.3	0.7			0.8	0.1	1.4	<0.1	334 (62)
American shad	*Alosa sapidissima*									0.4	0.5	154 (42)
Atlantic croaker	*Micropogonias undulatus*	14.7	1.5	0.8	1.4	5	1.3	1.7	0.6	3.4	0.4	126 (51)
Atlantic herring	*Clupea harengus*			7.3	10.4					1.4	0.2	217 (24)
Atlantic menhaden	*Brevoortia tyrannus*	72.4	17.8	82.8	60.4	71.1	4.9	94.5	81.8	67.9	17.3	183 (73)
Bay anchovy	*Anchoa mitchilli*	6.7	63.4	0.9	11.8	11.9	92.1	0.2	12.6	16.5	68.6	55.9 (13)
Blueback herring	*Alosa aestivalis*									1.1	1.7	99.1 (67)
Butterfish	*Peprilus triacanthus*			3.1	2.8			0.5	3.41	0.3	0.4	179 (35)
Hickory shad	*Alosa mediocris*					5.4	0.1			0.3	<0.1	442*
Round herring	*Etrumeus teres*					3.5	0.5			0.5	0.5	135 (9)
Sciaenid sp.										1.9	3.7	85.9 (28)
Silver perch	*Bairdiella chrysoura*			0.6	2.1	0	0			0.1	0.1	112*
Spot	*Leiostomus xanthurus*			0.5	0.7	2	0.6	<0.1	0.1	0.1	0.3	122 (14)
Tonguefishes	*Symphurus* sp.	5.9	14.9							0.5	0.4	
Unknown clupeid										1.1	0.9	391 (1.4)
Fish remains		0.4	2.5	0.1	2.1	0.2	0.9	1	0.9	1.5	1.2	
Weakfish	*Cynoscion regalis*			3.6	6.3	0.4	0	1.1	0.3	0.7	0.2	159 (15)
Menticirrhus spp.						0.4	0.1	0.3	0.1	0.2	0.1	137 (8.3)
Invertebrates												
Gastropod shell	Gastropod									0.2	0.7	
Decapods	Decapoda									0.3	0.6	
Sand shrimp	*Crangon septimspinosa*									0.4	1.3	
Mud crab	*Panopeus herbstii*			<0.1	0.7	0.1	0.1			<0.1	<0.1	
Longfin squid	*Loligo pealeii*					<0.1	<0.1			<0.1	<0.1	

(>70%) from 2005 through 2007 and was highest in 2007 (94.5%). Contribution of Atlantic menhaden by number, however, was quite variable: in 2005 they contributed 60.4%, declining to <5.0% in 2006, and increasing to 81.1% in 2007. Percent by number of bay anchovy was variable during 2005–07. In 2005 and 2007 their contribution by number was <15%. However, in 2006 they contributed >90% to the diet numerically.

Weakfish and Atlantic croaker were present in stomachs of recreationally caught striped bass for all years (Table 2). There were no clear patterns to their contributions, and collectively represented a small portion <5.0% (biomass) of the diet. Only one alosine, a hickory shad (2006), was found in the samples (2.7% biomass, 0.1% number). Other clupeoids, including Atlantic herring (7.3% of the biomass in 2005) and round herring (*Etrumeus teres*) (3.5% of the biomass in 2006), were found sporadically in the diet. Invertebrates were unimportant to the diet of large striped bass, generally representing <1.0% by weight and by number in the diet (Table 2).

Predator-prey size relationships

Prey consumed by striped bass ranged from 35 to 423 mm TL (mean=102.5 mm ±79.3 SD), although most prey items (86.7%) were less than 125.0 mm long. Mean length of Atlantic menhaden consumed was 204.2 TL mm (±76.2 mm SD). Prey length showed a significantly positive relationship with striped bass total length ($P<0.001$, $r^2=0.31$) (Fig. 4). The distribution of prey-to-predator ratios (PPR) ranged from 0.02 to 0.43 but had a skewed distribution toward the lower end of the range (75% of PPRs <0.15) (Fig. 5). This PPR distribution had a bimodal pattern with peaks at 0.07 and 0.14. Mean PPR for all prey was 0.12 (±0.07 SD) but the mean PPR for Atlantic menhaden was 0.19 and for bay anchovy, 0.06.

Discussion

This study concentrated on migratory adult striped bass that reside in nearshore waters of the Atlantic Ocean

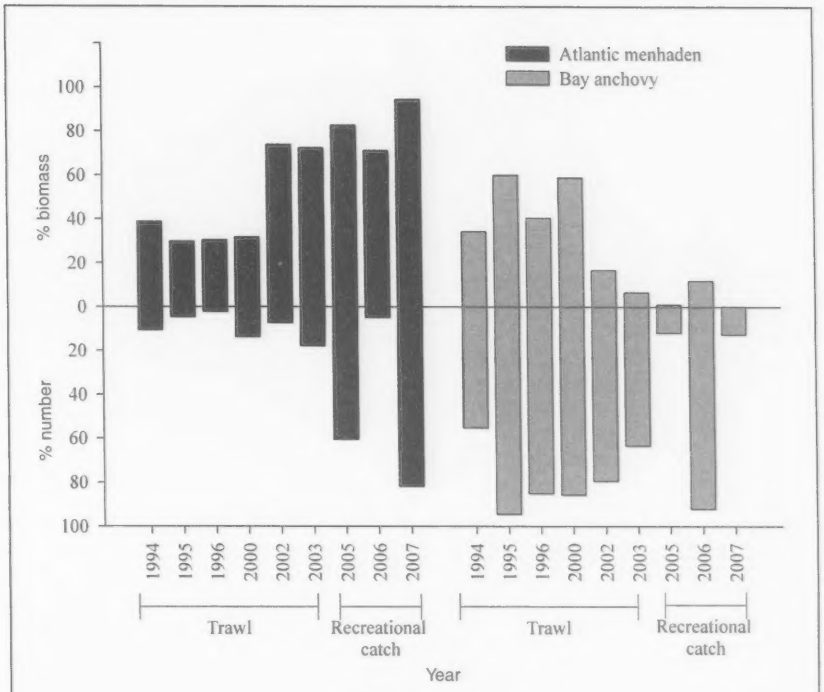

Figure 3

Percent biomass and number of Atlantic menhaden (*Brevoortia tyrannus*) and bay anchovy (*Anchoa mitchilli*) in the diet of striped bass (*Morone saxatilis*) collected in trawl and recreational catch samples during winter off the coasts of Virginia and North Carolina during 1994–2007.

off the coasts of Virginia and North Carolina in winter. We present the first published description of the diet of large striped bass, generally >400 mm TL, during their ocean residency in winter. The predominance of fish in the diet of striped bass in this study agrees with the findings of other published studies (Manooch, 1973; Overton, 2003; Walter et al., 2003; Walter and Austin, 2003). Several species of clupeoid fishes (e.g., Atlantic menhaden, Atlantic herring, and bay anchovy) dominated diet biomass of striped bass. This dependency on clupeoids, particularly Atlantic menhaden, has been well-documented throughout the range of striped bass (Walter et al., 2003). The only other study to address the diet of striped bass >500 mm TL was conducted in Chesapeake Bay by Walter and Austin (2003). They showed that Atlantic menhaden contributed 58% of the diet biomass. In the present study, Atlantic menhaden represented a higher biomass (67.9%) of the striped bass diet, indicating a greater dependency on Atlantic menhaden during the period of ocean residence in winter.

Anadromous species, particularly alosines, contribute substantially to the diet of striped bass (Nelson et al., 2003; Walter et al., 2003; Savoy and Crecco, 2004). In our study, there were less than five occurrences of alosines, which would indicate that anadromous alosines contribute little to the production of striped bass during their ocean residency in winter. Striped bass share similar migration patterns of other anadromous species (Walter et al., 2003) and we commonly observed alosines in the same trawls in which striped bass were collected.

Invertebrates were seemingly unimportant to large striped bass winter production because they contributed little to the diet. However, throughout their range, large striped bass routinely feed on a wide variety invertebrate prey. In New England waters during summer and fall, striped bass consumed large amounts of invertebrate prey such as sand shrimp (*Crangon septemspinosa*), rock crabs (*Cancer irroratus*), and American lobster (*Homarus americanus*) (Nelson et al., 2003). Large striped bass in Chesapeake Bay routinely fed on invertebrate prey, primarily blue crab (*Callinectes sapidus*), in summer (Walter and Austin, 2003). Presumably, these differences among studies due to differences in prey availability.

The percentage of stomachs with food varied among years but ranged from 23% in 2005 to 100% in 1995. In Chesapeake Bay, the percentage of large striped bass with food in their stomach during fall and early winter (November and December) was greater than 75%

(Walter and Austin, 2003). Similarly, the percentage of striped bass stomachs with food during winter in our study was generally greater than 75%.

Less than 30% of stomachs sampled during 2005–06 from the recreational catch contained prey, but 80% did during 2007. Striped bass likely expel some stomach contents while being reeled to the surface or while in the codend of a trawl. We did not determine the factors that influence regurgitation with respect to capture method. The alimentary canal musculature is stronger in larger fish and would result in lower regurgitation rates (Staniland et al., 2001). Regurgitation of stomach contents from striped bass collected by hook and line generally consisted of slurry (Overton, 2003). Regurgitation rates for adult striped bass captured in gillnets was 8.3% (Sutton et al., 2004). For 2007, the percentage of stomachs with food was greater than 80%. The high frequency of nonempty stomachs in this study may indicate that the winter feeding period for the migratory stock may play an important role in providing energy for growth and gonadal development.

Striped bass consumed small prey and the mean size of prey consumed was 12% of their total length and ranged from 2% to 43%. This mean percentage was lower than the predicted optimal size of prey (21%) predicted for striped bass (Overton, 2003), but was within the range of the predicted minimum profitable prey lengths (7%), peak profitable (12%) and maximum (40%) for striped bass (Hartman, 2000). In Albemarle Sound, North Carolina, striped bass consumed prey up to 60% of their body length, although mean prey size consumed was 20% of body length (Manooch, 1973). In a more recent study, age 1–3 striped bass in Albemarle Sound, North Carolina, on average consumed prey about 21% of their body length (Rudershausen et al., 2005). Piscivores generally select for smaller-size prey (Juanes and Conover, 1994). The differences among the studies indicate that larger striped bass include smaller prey in their diet. It may also indicate that there are fewer large prey available to striped bass during the winter. However, we observed that the finfish bycatch during the striped bass survey comprised prey larger than what was observed in the stomachs of striped bass.

There was a significant positive relationship between prey size and predator size which suggests that larger striped bass consumed larger prey. Nevertheless, the fit of the regression was weak (r^2=0.31), indicating a wide variation of prey size was included in the diet. The invertebrate prey in the stomachs was generally <5.0% of the predator total length. About 75% of all prey consumed were less than 15% of the total predator length. These percentages were primarily driven by the large number of bay anchovy consumed by striped bass. The average size of Atlantic menhaden that were found in the stomach of striped bass was 204 mm TL. Atlantic menhaden undergo an extensive coastal migration southward around the Virginia and North Carolina capes in fall and winter (Reintjes and Pacheco, 1966). All ages in the population participate in this migration; however, younger fish tend to be found within a few miles of the shoreline, while older individuals may be found farther offshore (Reintjes and Pacheco, 1966). Thus, the age-specific distribution of Atlantic menhaden probably influences prey-size availability to striped bass in nearshore ocean waters. In turn, striped bass potentially have significant impacts through the reduction of age-0 fish on the spawning stock of Atlantic menhaden.

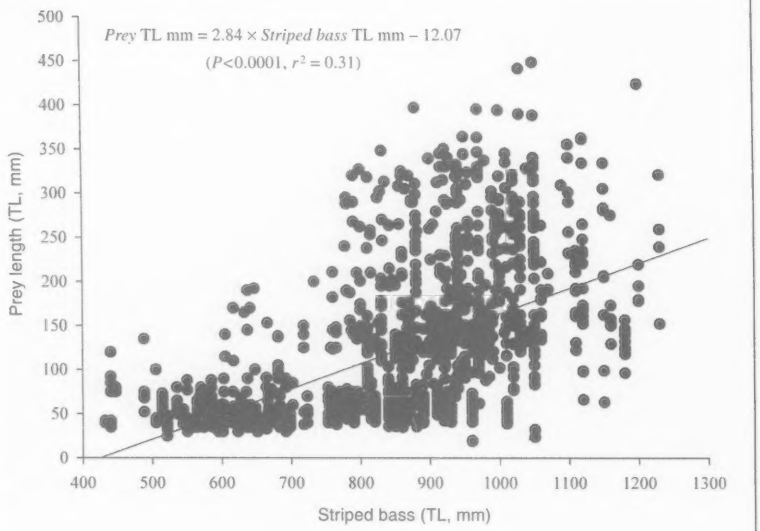

Figure 4

Relationship between striped bass (*Morone saxatilis*) total length (TL, mm) and prey length (prey TL mm=–12.07 + 2.84 (striped bass TL); P=<0.0001, r^2=0.31)

The frequency of Atlantic menhaden in the diet has increased from 1997 through 2007 and likely represents an increase in the competition between other predators and the existing commercial fishery (Uphoff, 2003). Given that Atlantic menhaden provide up to 60% of the diet for age 3+ striped bass in Chesapeake Bay (Hartman and Brandt, 1995) and 69% of the diet for striped bass in this study, and given the increased population levels of striped bass, it is likely that striped bass predation represents a large part of the natural mortality for Atlantic menhaden (Hartman, 2003; Hartman and Margraf, 2003; Uphoff, 2003).

Coastwide population-level consumption of Atlantic menhaden by striped bass in the Atlantic Ocean increased from 50×10^3 t in 1982 to over 250×10^3 t in 2000 (Overton, 2003). Striped bass are capable of exerting considerable pressure on prey populations through predation (Hartman, 2003; Grout, 2006). With concerns over Atlantic menhaden recruitment, it is essential to quantify its role as a prey fish and its major sources of mortality.

Our diet data were collected by using two different methods during two separate time periods; therefore we were unable to test the effects of collection methods on the diet composition. Nevertheless, we feel that the two collection methods complement each other. For example, the trawl samples (1994–2003) show an increasing trend in the amount of Atlantic menhaden consumed; these data are supported by the recreational catch data. Simultaneously, the trawl data show a decline in the consumption of bay anchovy diet, which is also supported by the recreational catch data. These results indicate that the recreational catch data provide a reasonable representation of the diet of striped bass during the winter off the coasts of North Carolina and Virginia.

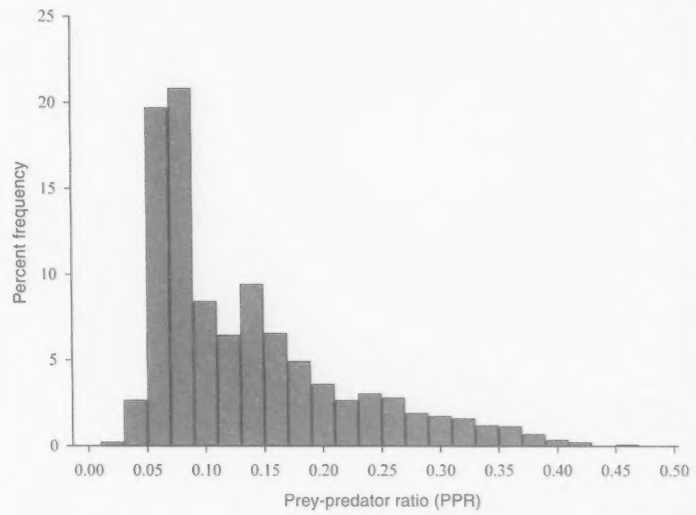

Figure 5

Prey-to-predator ratio (PPR) frequency distributions determined from the diet of striped bass (*Morone saxatilis*) collected in trawl and recreational catch samples during winter off the coasts of Virginia and North Carolina during 1994–2007.

To further understand the predator-prey interactions of striped bass, we suggest a continued low-frequency monitoring of predator diets along the Atlantic coast. Low-frequency monitoring approaches have been used to estimate the consumption of commercially important fish by predatory fish in the western North Atlantic and can provide important insights regarding the importance of prey types (Overholtz et al., 2000). These data can be used to calibrate different predator-prey, bioenergetic, and multispecies models for different management systems. This information could provide data that would add significantly to knowledge of trophic interactions of striped bass and other predators.

This analysis of the foraging behavior of large migratory striped bass during their winter residency in the Atlantic Ocean contributes to the increasing literature on the foraging dynamics of predatory fishes. Whether the patterns observed during our study period were the result of prey dynamics or predator function is unclear. However, striped bass feed on a large number of prey during winter and are also capable of feeding on a wide range of prey sizes. This work outlines the importance of clupeoid fishes to striped bass winter production and also shows that predation may be exerting prey pressure on Atlantic menhaden stocks.

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Identification of larval sea basses (*Centropristis* spp.) using ribosomal DNA-specific molecular assays*

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Abstract—The identification of sea bass (*Centropristis*) larvae to species is difficult because of similar morphological characters, spawning times, and overlapping species ranges. Black sea bass (*Centropristis striata*) is an important fishery species and is currently considered to be overfished south of Cape Hatteras, North Carolina. We describe methods for identifying three species of sea bass larvae using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays based on species-specific amplification of rDNA internal transcribed spacer regions. The assays were tested against DNA of ten other co-occurring reef fish species to ensure the assay's specificity. *Centropristis* larvae were collected on three cruises during cross-shelf transects and were used to validate the assays. Seventy-six *Centropristis* larvae were assayed and 69 (91%) were identified successfully. DNA was not amplified from 5% of the larvae and identification was inconclusive for 3% of the larvae. These assays can be used to identify sea bass eggs and larvae and will help to assess spawning locations, spawning times, and larval dispersal.

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A fundamental requirement of early life stage studies is the ability to identify individuals to species. Considerable knowledge of the eggs and larvae of most fish species in temperate and boreal ecosystems has been achieved (Kendall and Matarese, 1994; Berrien and Sibunka, 1999). In contrast, in subtropical and tropical ecosystems, the larvae of many species cannot be identified (Kendall and Matarese, 1994), even for groups of important fishery species. For example, of the 73 species in the snapper-grouper complex that range within U.S. federal waters, larvae of only one-half can be identified to species and eggs of most species are undescribed (Richards, 2006).

As anthropogenic stress on marine ecosystems continues to increase, new approaches to early life stage identification are needed to obtain critical information about exploited species and to improve the scientific basis for conservation. For example, immunological methods have been used successfully to identify invertebrate and fish larvae (Miller et al., 1991; Paugam et al., 2000; Garland and Zimmer, 2002; Taylor, 2004). Similarly, mitochondrial DNA sequences (Hare et al., 1994; Pegg et al., 2006) and restriction fragment length polymorphisms (RFLP) have been used to identify fish eggs, larvae, and adults (Daniel and Graves, 1994; Aranishi et al., 2005; Hyde et al., 2005). Though highly accurate, these methods have yet to be used widely as part of standard protocols for identifying fish larvae collected during research and monitoring surveys.

We examined the application of molecular techniques for identifying species within the genus *Centropristis* (sea bass). Five species of *Centropristis* are currently recognized in the western North Atlantic Ocean. In addition to black sea bass (*C. striata*), there are the following: bank sea bass (*C. ocyurus*), rock sea bass (*C. philadelphica*), twospot sea bass (*C. fusculа*), and *C. rufus*. Black sea bass is an important commercial and recreational species on the northeast and southeast U.S. continental shelf. Along

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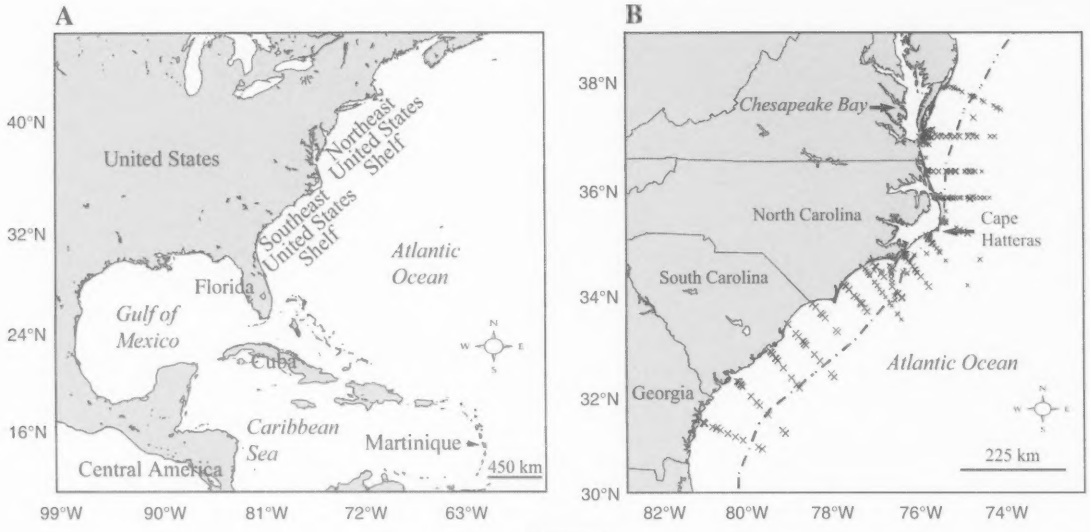

Figure 1

(**A**) Five species of *Centropristis* (sea bass) are currently recognized in the western North Atlantic Ocean. Black sea bass (*Centropristis striata*) ranges along the northeast and southeast shelves of the U.S. coast and inhabits the Gulf of Mexico on the northwest coast of Florida. Bank sea bass (*C. ocyurus*) and rock sea bass (*C. philadelphica*) are found along the southeast U.S. coast and in the Gulf of Mexico. *Centropristis rufus* is reported off Martinique in the southeastern Caribbean Sea. Twospot sea bass (*C. fuscula*) is reported from Cuba. (**B**) Sampling stations are marked with an x where *Centropristis* spp. larvae were collected during three cruises ranging from Chesapeake Bay to southern Georgia during September 2000, November 2000, and February through March 2001. During each cruise, transects running cross-shelf from 10 m to > 1000 m water depth were sampled. The dashed line approximates the 200-m depth contour.

the southeast U.S. shelf and the Gulf of Mexico where *C. striata*, *C. ocyurus*, and *C. philadelphica* cohabit (Fig. 1), larvae less than 12 mm cannot be reliably identified to species because fin ray formation is not complete. To identify larvae of these species, we developed species-specific polymerase chain reaction (PCR) and RFLP assays based on the internal transcribed spacer (ITS) regions of the ribosomal gene complex. The ITS regions are noncoding spacer regions situated between the 18S and 5.8S rRNA genes (ITS1) and between the 5.8S and 28S rRNA genes (ITS2) (Fig. 2A). The ITS regions work well for species identification because in most eukaryotic organisms these regions diverge rapidly during speciation, enabling even closely related species to be distinguished. The ITS sequences have been used to successfully identify species in groups as diverse as fungi (Lu et al., 2002), plants (Baldwin, 1992), insects (Rafferty et al., 2002), and dinoflagellates (Connell, 2001; Litaker et al., 2003). Other ITS-based assays have been developed to help address conservation and management problems in the trade of shark products (Chapman et al., 2003; Shivji et al., 2005) and for identifying wildlife tissues in forensic applications (Sweijd et al., 2000; Ambercrombie et al., 2005).

PCR and RFLP assays offer rapid methods for identification of fish larvae and eggs. The PCR assays can be performed in a one-step process after DNA extraction and they enable species identifications to be made even when meristic characters and other identifying features have been damaged or are underdeveloped. Alternatively, the RFLP assays provide a cost efficient, two-step, PCR-based species identification method. In the first step, PCR is used to amplify the ITS regions. In the second step, restriction enzyme digestion of the PCR product is conducted and species-specific fragments are analyzed. This study describes the development, optimization, and validation of PCR and RFLP assays for three species of *Centropristis*.

Materials and methods

Collection and preservation of adult fish

Tissue samples were obtained from adult and juvenile fish and either stored in 95% ethanol or frozen at –80°C. *Centropristis striata* were collected from the Atlantic coast of the southeast United States, and *C. ocyurus* and *C. philadelphica* were collected from both the southeastern U.S Atlantic coast and the Gulf of Mexico. At least three individuals from each location were analyzed. Ten other co-occurring southeast U.S. reef fishes were also collected for sequencing of the ITS regions and served as negative controls for the PCR and RFLP assays. Five of these species were from the subfamily Serraninae: sand perch (*Diplectrum formosum*), butter hamlet (*Hypoplec-*

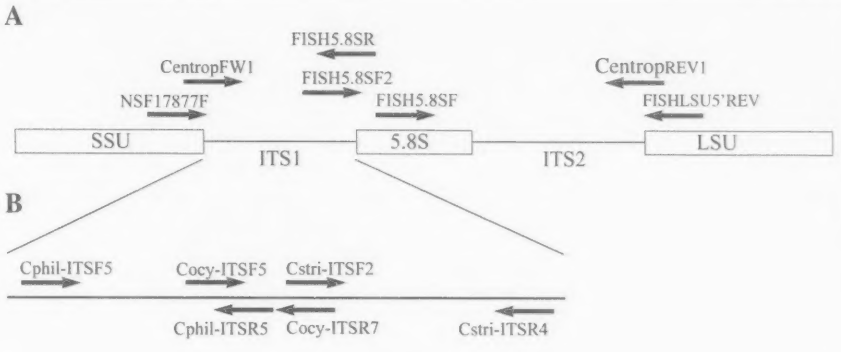

Figure 2

Diagram of the small subunit (SSU), internal transcribed spacer regions (ITS), 5.8S, and large subunit (LSU) genes. (**A**) Location of the universal polymerase chain reaction (PCR) primers used to amplify the ITS spacer regions, the genus-specific restriction fragment length polymorphism (RFLP) primers, and the internal sequencing primers. (**B**) Location in the ITS1 region of the *Centropristis* PCR assay primers.

trus unicolor), pygmy sea bass (*Serraniculus pumilio*), tattler (*Serranus phoebe*), and belted sandfish (*Serranus subligarius*). Four species were from the family Serranidae, red grouper (*Epinephelus morio*), rock hind (*Epinephelus adscensionis*), speckled hind (*Epinephelus drummondhayi*), gag grouper (*Mycteroperca microlepis*), and one from a related perciform family, Sparidae, the spottail pinfish (*Diplodus holbrooki*).

Collection and preservation of larvae

Three cruises were conducted from Chesapeake Bay to southern Georgia during early fall (September 2000), late fall (November 2000), and late winter (February and March 2001). During each cruise, transects running cross-shelf from 10 m to >1000 m water depth were sampled (Fig. 1). The deepest stations were sampled to 50 m. Ichthyoplankton were collected with a 1-m Tucker trawl fitted with 333-μm mesh nets. The Tucker trawl was fitted with three nets. The first net sampled from the surface to the deepest deployment depth. For stations in <50 m water depth, the lower and upper halves of the water column were sampled discretely by using the second and third nets during the retrieval of the Tucker trawl. For stations in >50 m water depth, the 50–25 m depth was sampled discretely with the second net and the 25–0 m depth interval was sampled discretely with the third net. Depth was determined from the wire angle of the gear and length of wire deployed. Tow speed was between 1.5 and 2 knots.

Samples were initially preserved in 95% ethanol. All larvae were sorted from these samples, transferred to 70% ethanol, and identified to the lowest taxonomic level possible. Larvae were identified to the genus *Centropristis* based on characteristic body shape, pigmentation, and when available, dorsal and anal fin meristics (Kendall, 1972). Seventy-six larvae were chosen for genetic analyses. These specimens were selected in an attempt to analyze equal numbers of larvae collected from north and south of Cape Hatteras, North Carolina. In this respect, the larvae were not a random sample of the *Centropristis* larvae identified from the collections. The larvae ranged in size from 1.5–11 mm notochord length (or standard length). Each individual to be used for PCR analyses was rinsed three times with clean 95% ethanol, digitally imaged, and stored in a separate sterile vial with 95% ethanol. To prevent DNA cross-contamination, the forceps used for handling larval fish were decontaminated between specimens with 10% sodium hypochlorite, rinsed with clean deionized water, and dried with a clean Kimwipe (Kimberly-Clark, Roswell, GA).

Determination of ITS region sequences

To identify species-specific primer sites it was first necessary to amplify and sequence the ITS regions from adult *Centropristis* and other reef fish. To accomplish this, universal PCR primers were designed for the amplification of the 3′ end of the small subunit (SSU), the internal, transcribed spacer region (ITS1), 5.8S subunit (5.8S), the second internal transcribed spacer region (ITS2), and the first ~48 base pairs of the large subunit (LSU) rRNA genes (~1300-bp product). The universal forward primer NSF1787F (Fig. 2A, Table 1) was designed with the reverse complement of the universal reverse primer, NSR1787/18, listed on the ribosomal RNA database website. The universal reverse primer FishLSU5'Rev (Fig. 2A, Table 1) was designed by aligning (CLUSTAL X algorithm; Thompson et al., 1997) the large subunit rDNA sequences of the following fish species that were available in GenBank: European perch (*Perca fluviatilis* Z18686), rainbow trout (*Oncorhynchus*

mykiss OMU34341, Z18683), tub gurnard (*Trigla lucerna* Z18768), Atlantic mackerel (*Scomber scombrus* Z18693), zebrafish (*Brachydanio rerio* AJ306603), *Pimelodella cristata* (AJ306596), Atlantic herring (*Clupea harengus* Z18764), dab (*Limanda limanda* Z18681), brown bullhead (*Ictalurus nebulosus* Z18678), spotted green pufferfish (*Tetraodon nigroviridis* AJ270038, AJ270037), common carp (*Cyprinus carpio* AF133089), black ghost (*Apteronotus albifrons* AJ306595), Eurasian minnow (*Phoxinus phoxinus* AJ306604), *Synodontis clarias* (AJ306597), *Orinocodoras eigenmanni* (AJ306606), angler (*Lophius piscatorius* Z18765), European pilchard (*Sardina pilchardus* Z18767), coelacanth (*Latimeria chalumnae* U34336), ghost shark (*Callorhinchus milii* AY049812), Florida gar (*Lepisosteus platyrhynchus* Z18680), arawana (*Osteoglossum* sp. Z18684), rabbit fish (*Chimaera monstrosa* Z18674), European eel (*Anguilla anguilla* Z18673), bowfin (*Amia calva* Z18672), and browneye skate (*Raja schmidti* AF278683).

DNA purification

For adult specimens, approximately 50–100 mg of muscle tissue or a clipping from the fin was collected from either frozen or ethanol-preserved specimens and DNA was extracted with a Roche High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's protocol for isolation of nucleic acids from mammalian tissue. The one exception to the manufacturer's protocol was that we used a volume of 50 μL to elute the DNA in the final step of the procedure. For ethanol-preserved specimens, the tissues were rehydrated in three changes of sterile tris ethylenediaminetetraacetic acid (TE) buffer for 30 minutes each. Tissues were then transferred to the cell lysis solution, and DNA was purified following the manufacture's protocol.

DNA extraction from larval fish was accomplished by first placing them in separate sterile 55 mm Petri dishes and allowing them to rehydrate in sterile TE buffer [10mM Tris HCl (pH 7.4), 1mM disodium ethylenediaminetetraacetate (EDTA) (pH 8.0)] for 30 minutes. Next, an eye was microdissected with fine forceps and the DNA was purified as described above. This limited the physical damage to the larvae, allowing them to be used for future meristic studies. The forceps used for microdissection were decontaminated between specimens as described above.

PCR amplification, cloning, and sequencing procedures for ITS regions of adult fish

The PCR amplification reaction mixture used to amplify adult fish ITS regions is listed in Table 2 . DNA was PCR amplified in a MJ Research MiniCycler (MJ Research, Waltham MA) under the following touchdown cycling conditions: 2 min. at 95°C, 35 cycles each consisting of 40 sec. denaturation at 95°C, 40 sec. initial annealing temperature at 64°C which decreased by 0.5°C per cycle for six cycles and 61°C thereafter and an extension of 1.5 min. at 72°C. This procedure was followed by a final extension of 5 min. at 72°C. A 4-μL aliquot of each PCR reaction was checked for the presence of a specific amplification product by agarose gel electrophoresis (2% agarose, tris acetate EDTA [TAE gel], 50 volts) and ethidium bromide staining. PCR reactions containing specific products were cloned into the plasmid vector pCR2.1 by using the Topo TA Cloning Kit and by following the manufacturer's protocol (Invitrogen, Carlsbad, CA). Plasmids were isolated and purified with a QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA) and sequenced with an ABI377 DNA sequencer employing the Deoxy Terminator Cycle sequencing kit (Applied Biosystems–ABI, Foster City, CA). In addition to the ten local reef fishes, three adult fish of each *Centropristis* species were PCR amplified and cloned. DNA templates were sequenced completely in both directions with the M13F, M13R, Fish5.8SF, Fish5.8SF2, and Fish5.8SR primers (Fig. 2A, Table 1). The resulting 3′ SSU to 5′ LSU sequence for each species was assembled by using the Vector NTI program (Informax Inc., Bethesda, MD) and submitted to GenBank (EF472464–EF472500).

Table 1

Primers used in this study to amplify and sequence the 3′ end of the small subunit gene, internal transcribed spacer 1, 5.8S gene, internal transcribed spacer 2, and the first approximately 48-bp of the large subunit gene

Primer name	Sequence (5′–3′)
Forward sequencing	
M13F (plasmid vector)	GTAAAACGACGGCCAG
NSF1787F (universal forward)	CCGTAGGTGAACCTGCGG
Fish5.8SF	AGCTGCGAGAACTAATGTGAA
Fish5.8SF2	TGCTCTGCTCGGGCTGTAGCG
Reverse sequencing	
M13R (plasmid vector)	CAGGAAACAGCTATGAC
FishLSU5'Rev (universal reverse)	CTTAAATTCAGCGGGTTGTCT
Fish5.8SR	TTCACATTAGTTCTCGCAGCT

Phylogenetic analysis

The ITS rDNA gene sequence from *C. striata*, *C. ocyurus*, *C. philadelphica*, and *Diplectrum formosum* (for an outgroup) were aligned using the CLUSTAL-X algorithm (Thompson et al., 1997). A Bayesian phylogenetic analysis of the aligned sequences was performed with the MrBayes 3.1 program (Huelsenbeck et al., 2001). Posterior probabilities were calculated by using a Metropolis-coupled Markovian Chain Monte Carlo approach and with sampling conducted according to the Metropolis–

Table 2

Polymerase chain reaction (PCR) reagent concentrations used amplify fish internal transcribed spacer regions and conduct PCR and restriction fragment length polymorphism (RFLP) assays for *Centropristis striata* (black sea bass), *C. ocyurus* (bank sea bass), and *C. philadelphica* (rock sea bass).

Reagent	Fish ITS PCR reaction mix	*C. striata* assay	*C. ocyurus* assay	*C. philadelphica* assay	RFLP PCR reaction mix
Tris-HCl	100 mM, pH 8.8	100 mM, pH 8.8	200 mM, pH 8.4	100 mM, pH 8.8	100 mM, pH 8.3
$MgCl_2$	15 mM	15 mM	30 mM	15 mM	15 mM
KCl	750 mM	750 mM	500 mM	750 mM	750 mM
Primer I	25 pmoles	25 pmoles	25 pmoles	25 pmoles	25 pmoles
Primer II	25 pmoles	25 pmoles	25 pmoles	25 pmoles	25 pmoles
dNTP mix	10 mM	10 mM	10 mM	10 mM	10 mM
DMSO	5%	5%	5%	5%	5%
Taq DNA polymerase	0.25 units	0.25 units	0.25 units	0.25 units	0.25 units
DNA template	20–50 ng	20–50 ng	20–50 ng	20–50 ng	20–50 ng
Reaction volume	50 μL	50 μL	50 μL	50 μL	50 μL

Table 3

Primer pairs used to conduct the *Centropristis* (sea bass) polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays. bp=base pairs.

Species	Primer	Primer sequence 5′–3′	PCR product size
C. striata assay	Cstri-ITSF2 (forward)	TGGACCGGCTTTCCTCCCG	230 bp
	Cstri-ITSR4 (reverse)	CAATGAGGGTTGGAGAAAGGG	
C. ocyurus assay	Cocy-ITSF5 (forward)	CTCGTCCTCCTTGCGGTGG	158 bp
	Cocy-ITSR7 (reverse)	GGAGGTTTTGGTTTGTGTAGG	
C. philadelphica assay	Cphil-ITSF5 (forward)	CACTGCCACTGCCTCCAC	238 bp
	Cphil-ITSR5 (reverse)	ACGGAGCCAGCTTTCACC	
Centropristis RFLP assay	CentropFW1 (forward)	GATCATTACCGGTCGGTTGC	~1200 bp
	CentropREV1 (reverse)	GTAGTCGAAAAGTGGAGGCAG	

Hastings algorithm. The phylogenetic analysis employed two concurrent analyses of four chains each. In each analysis, one cold and three incrementally heated chains were used where the heat of the ith chain is $B = 1/[1+(i-1)T]$ and $T = 0.2$. Initial phylogenetic trees for each chain were random and used default starting values of MrBayes. A single run consisting of 100,000 generations was conducted. The run was sampled at every 100th tree. Only trees sampled after a stable burn-in of 1000 generations were used. The results were plotted as a rooted phylogram with *Diplectrum formosum* as the outgroup. An outgroup is any species occurring outside a particular branch or clade, i.e., a species that is further towards the root of a phylogenetic tree.

Development of species-specific PCR assays for identifying larval fish

The 3′ SSU – 5′ LSU sequences for *Centropristis striata* (EF472473-EF472481), *C. philadelphica* (EF472482-EF472490), *C. ocyurus* (EF472491-EF472499), *Diplectrum formosum* (EF472472), *Diplodus holbrooki* (EF472471), *Epinephelus morio* (EF472468), *Epinephelus adscensionis* (EF472470), *Epinephelus drummondhayi* (EF472469), *Hypoplectrus unicolor* (EF472467), *Mycteroperca microlepis* (EF472466), *Serraniculus pumilio* (EF472465), *Serranus phoebe* (EF472464), and *Serranus subligarius* (EF472500) were aligned by using the CLUSTAL-X algorithm. The alignments were used to identify unique ITS sequences and to develop species-specific PCR assays. For all three *Centropristis* assays, we used forward and reverse primers located within the ITS1 region (Fig. 2B). The PCR primers used for each assay are listed in Table 3. The assays were conducted under touchdown PCR cycling conditions with an MJ Research PTC-150 MiniCycler. The PCR reaction profile for the *C. striata* assay was as follows: 2 min. at 95°C, 35 cycles each consisting of 30 sec. denaturation at 95°C, 40 sec. initial annealing temperature at 66°C which decreased by 0.5°C per cycle for four cycles and 64°C thereafter, and

an extension of 45 sec. at 72°C. This was followed by a final extension of 5 min. at 72°C. The cycling conditions for the *C. ocyurus* assay were the following: 2 min. at 95°C, 35 cycles each consisting of 30 sec. denaturation at 95°C, 40 sec. initial annealing temperature at 64°C which decreased by 0.5°C per cycle for four cycles and 62°C thereafter and an extension of 45 sec. at 72°C. This was followed by a final extension of 5 min. at 72°C. Lastly, the cycling conditions for the *C. philadelphica* assay were: 2 min. at 95°C, 35 cycles each consisting of 30 sec. denaturation at 95°C, 40 sec. initial annealing temperature at 60°C which decreased by 0.5°C per cycle for four cycles and 58°C thereafter and an extension of 45 sec. at 72°C. This was followed by a final extension of 5 min. at 72°C. An aliquot (4 μL) from each amplification was analyzed by agarose gel electrophoresis (3% agarose TAE gel, 75V). The sizes of the PCR products were estimated with either a 123-bp or 50-bp ladder (Promega, Madison, WI).

Validation of the PCR assays for identifying larval fish

Following assay development, the primer pairs were tested for cross-reactivity with a panel of DNAs that included the 10 reef fish species listed previously (20–50 ng of DNA per PCR reaction). DNA from 76 *Centropristis* larvae was extracted using the methods described above. The larvae were assayed by using 20–50 ng of DNA from each specimen. Each PCR assay included a positive control, a negative control, a blank DNA extraction control, and two PCR inhibition controls. The positive control incorporated 20 ng of appropriate *Centropristis* DNA in the reaction mixture. The negative control substituted 1×PCR buffer for DNA to confirm that the reagents were not contaminated with target DNA. The blank extraction control was included to assess possible cross-contamination during the extraction procedures. The inhibition control consisted of spiking 20 ng of appropriate *Centropristis* DNA into two arbitrarily chosen larval fish DNA samples. The PCR inhibition controls confirmed that negative PCR reactions were due to the absence of appropriate *Centropristis* DNA and not from PCR inhibition. The *C. striata* and *C. ocyurus* assays were tested on all of the larvae in the collection. The *C. philadelphica* assay was applied by process of elimination to larvae that did not yield results from the *C. striata* or *C. ocyurus* assays.

Development of RFLP assays for identifying larval fish

Forward and reverse genus-specific primers for three species of *Centropristis* were designed from the 3′ SSU–5′ LSU sequence alignment described above. The forward primer, CentropFW1 (Table 3), overlapped the boundary of the 3′ end of the SSU rRNA gene and the 5′ end of the ITS1 spacer (Fig. 2A). The reverse primer, CentropRev1 (Table 3), overlapped the boundary of the 3′ end of the ITS2 spacer and the 5′ end of the LSU rRNA gene (Fig. 2A). The primer pair yielded an approximate 1200-bp PCR product and was tested for cross-reactivity against the 10 non-*Centropristis* reef fishes listed above. The RFLP-PCR reaction mix is listed in Table 2. Twenty to 50 ng of genomic DNA was used per PCR reaction. PCR amplifications were conducted with an MJ Research PTC-150 MiniCycler under the following cycling conditions: 2 min. at 95°C, 40 cycles each consisting of 30 sec. denaturation at 95°C, 40 sec. initial annealing temperature at 63°C which decreased by 0.5°C per cycle for six cycles and 60°C thereafter, and an extension of 1 min. at 72°C. This was followed by a final extension of 5 min. at 72°C. A 4-μL aliquot of each PCR reaction was checked for the presence of a specific amplification product by agarose gel electrophoresis (2% agarose TAE gel, 50 V) and ethidium bromide staining. The sizes of the PCR products were estimated by using the DNA molecular weight marker IX (Roche Diagnostics, Indianapolis, IN).

RFLP analysis of *Centropristis* PCR products was simulated with the RFLP analysis tool in the Vector NTI program (Informax Inc., Bethesda, MD) to identify species-specific RFLP patterns. The restriction enzyme *Alu* I (New England Biolabs, Beverly, MA) was selected on the basis of the distinct restriction patterns predicted for each *Centropristis* species. Restriction digests were performed in 30-μL reactions containing 3 μL of New England Biolabs 10× reaction buffer #3, 25 μL of PCR product, 2000 units of *Alu I* enzyme, and were incubated at 37°C for 2 hours. Restriction fragments were separated by gel electrophoresis on 3% agarose, Tris-Borate EDTA NuSieve 3:1 gels (Cambrex Bio Science Rockland, Inc., Rockland, ME) and fragments sizes were estimated by using a 50-bp DNA ladder (Promega, Madison, WI).

Results

Species-specific molecular assays based on differences in the ITS rDNA regions were developed successfully for the identification of *Centropristis* larvae. The approach we employed in developing the molecular assays was 1) to identify universal PCR primer sites that would amplify the ITS regions of many fish species; 2) to sequence the ITS regions from three *Centropristis* species and other co-occurring reef species; 3) to develop species-specific PCR and RFLP assays for the *Centropristis* species based on alignments of the resulting sequences; and 4) to use the assays to identify field-collected larval *Centropristis*.

The universal PCR primers (Table 1) were successful in amplifying the ITS regions of many reef fish species. The primers generated an approximate 1200–1300 base pair product that varied with the species. A phylogenetic analysis of the *Centropristis* ITS sequence alignments showed that between-species sequence divergence in this region was much greater than within-species divergence (Fig. 3). This variation made it possible to develop species-specific PCR assays for *C. striata*, *C. ocyurus*, and *C. philadelphica*. The primers were tested for cross-reactivity against ten other related reef fish

species and they did not amplify non-target species (Fig. 4). The accuracy of the species-specific assays was checked by PCR assaying 15 adult and juvenile sea basses (5 of each species). The PCR assays were successful in identifying the target species and did not cross-react among the three *Centropristis* species.

We validated the PCR assays by using them to assess the identity of 76 *Centropristis* larvae (Fig. 5). Sixty-nine of the 76 larvae (91%) were identified successfully: 33 *C. striata*, 32 *C. ocyurus*, and four *C. philadelphica*. DNA from four of the larvae failed to PCR amplify and in three cases the PCR assay results were inconclusive. The four DNA samples that failed to amplify were tested for PCR inhibition by spiking a small amount of target *Centropristis* plasmid DNA into PCR reaction mixes containing DNA from the larvae. These spiked controls all amplified, indicating that PCR inhibition was not a factor. DNA was re-extracted from the larvae and attempts were made to re-amplify it with the species-specific primers and the universal primers. Neither primer sets produced a PCR product, indicating that DNA degradation had occurred to the extent that amplifiable DNA could not be isolated from these four specimens.

The three inconclusive results were symptomatic of DNA cross-contamination between larval samples. Both the *C. striata* and *C. ocyurus* specific primer sets produced correct size PCR products for these samples. DNA from these larvae was re-extracted in an attempt to eliminate the cross-contamination, but multiple PCR assay attempts yielded the same ambiguous results. The issues of cross-contamination and methods to avoid it will be discussed below.

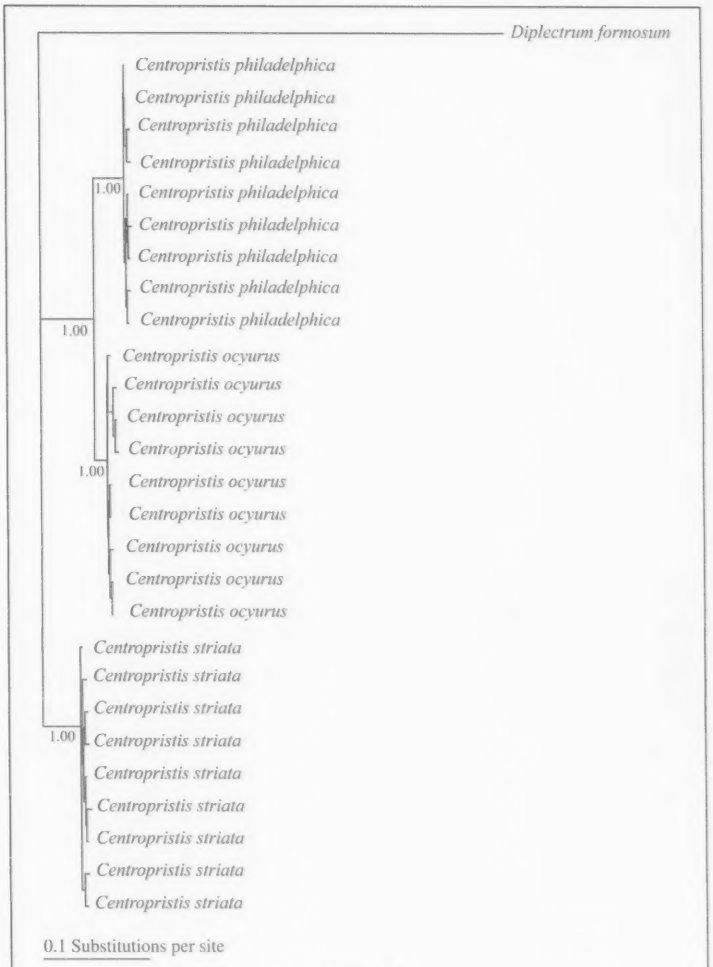

Figure 3

Phylogeny showing the relationship between black sea bass (*Centropristis striata*), bank sea bass (*C. ocyurus*), and rock sea bass (*C. philadelphica*) based on ITS1, 5.8S, and ITS2 rDNA sequences. Sand perch (*Diplectrum formosum*) served as the outgroup. Branch support for each species was 100% (1.0). The scale at the bottom of the phylogeny is proportional to the branch lengths in the phylogenetic tree that correspond to a divergence of 0.1 nucleotide substitutions per base pair in the DNA sequences. The within-species sequence variation was consistently less than that observed between species.

RFLP assays were also developed for each species as an alternative to the PCR assays. Genus-specific PCR primers (Table 3) that flanked the ITS regions were used to amplify DNA from identified adult sea basses. The genus-specific primer sites were located very near the universal primer sites but were designed to amplify only *Centropristis* DNA. The primers were tested for cross-reactivity against the same related reef species that were used in the PCR assays. Cross-reactivity did not occur (Fig. 6A). The PCR products were digested with the *Alu* I restriction enzyme. Electrophoresis yielded unique banding patterns (Fig. 6B). Digests of *C. striata* yielded fragments of 446, 418, 218, 125, and 4 base pairs. The restriction fragment sizes for *C. ocyurus* were 449, 362, 245, 120, 51 bp, and 4 base pairs. For *C. philadelphica* the fragments were 481, 261, 187, 185, 121, 58, and 4 base pairs. The advantages and disadvantages of this diagnostic approach are discussed below.

Discussion

Ribosomal DNA-specific assays for *Centropristis* larvae have proven to be robust and accurate. The difficul-

ties associated with identifying *Centropristis* larvae prompted this work. The ITS region was chosen for assay development because it diverges rapidly during speciation and contains unique primer sites for distinguishing species (Baldwin, 1992; Lu et al., 2002; Litaker et al., 2003). The divergence between *Centropristis* species was supported by the ITS phylogenetic analysis, which showed that *C. striata*, *C. ocyurus*, and *C. philadelphica* were genetically distinct from each other and from another related serranid species, *Diplectrum formosum* (Fig. 3). The analysis further showed that *C. ocyurus* and *C. philadelphica* are more closely related when compared to *C. striata*, which is consistent with the morphological characteristics of the adult fish. One of the goals of this study was to conduct the larval assays in a manner that would cause minimal damage to the preserved specimens. The method used in our study, where DNA was recovered from a single eye, made it possible to perform morphometric analyses on genetically identified fish and to evaluate whether species-specific characteristics exist that can be used to identify the larvae.

The PCR assays were successfully validated by using larvae that were collected on cruises ranging from Chesapeake Bay to southern Georgia during September 2000, November 2000, and February through March 2001 (Fig. 1). The peak spawning season for *C. striata* occurs between June and September in the Mid-Atlantic Bight (Able et al., 1995) and between March and May in the South Atlantic Bight (Wenner et al., 1986; Mercer, 1989; McGovern et al., 2002). Data for spawning of *C. philadelphica* comes from Miller (1959) who concluded that spawning occurs during May and June. Manooch (1984) indicated that ripe females of *C. ocyurus* were collected in the South Atlantic Bight during March and April and that young fish appear in late April, suggesting that spawning occurs offshore in spring. Results from our study confirmed that spawning of *C. ocyurus* overlapped with that of *C. striata* and occurred in September and as early as February to March. The data from our study for *C. philadelphica* are limited, making it difficult to determine whether the low abundance was due to spawning period, spawning locations, or spawning stock biomass. *Centropristis philadelphica* larvae may have been less abundant in the collections because most of the sampling was conducted in waters deeper than 10 m. Trawling studies off the southeastern United States indicate that *C. philadelphica* is a year-round resident of shallow coastal waters (<10 m) with sandy-mud substrates (Wenner and Sedberry, 1989) and is not present at greater depths over sandy-mud or hard bottoms (Wenner et al., 1979; Sedberry and Van Dolah, 1984). Furthermore, it is likely that the sampling of larvae did not occur during the peak spawning season for *C. philadelphica* (Miller, 1959).

Because PCR is a highly sensitive molecular technique, care must be taken to avoid contamination. The data indicated that DNA cross-contamination occurred in three of the larval samples. We hypothesize that the DNA contamination occurred while the *Centropristis* larvae were stored collectively or during the sorting of the larvae. Field-collected larvae should therefore be preserved individually and utensils or tools used to process larvae should be decontaminated between larvae during the sorting process with a reagent that destroys DNA.

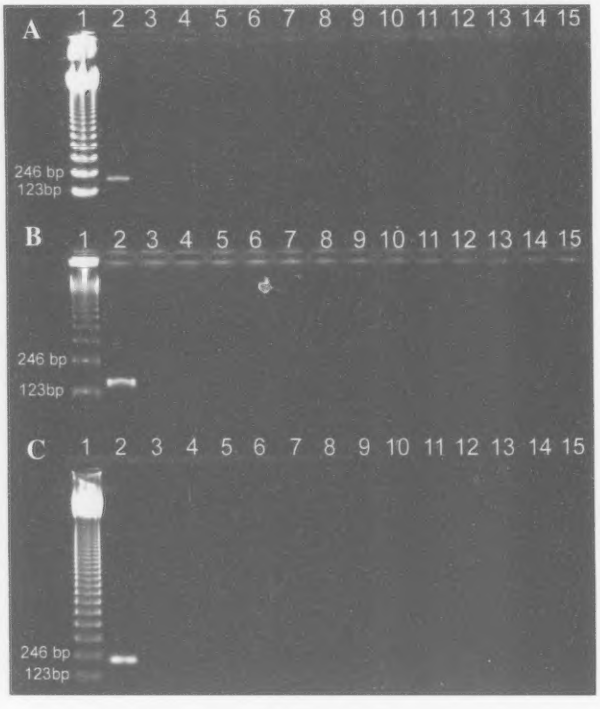

Figure 4

Results of cross-reactivity tests for (**A**) black sea bass (*Centropristis striata*), (**B**) bank sea bass (*C. ocyurus*), and (**C**) rock sea bass (*C. philadelphica*) polymerase chain reaction (PCR) assays. In each panel, the gels were loaded in the following order: lane 1, 123-bp ladder; lane 2, positive controls for A) *C. striata*, B) *C. ocyurus*, and C) *C. philadelphica*; lane 3, A) *C. ocyurus*, B) *C. striata*, C) *C. philadelphica*; lane 4, A) *C. philadelphica*, B) *C. striata*, C) *C. ocyurus*; lanes 5–14, sand perch (*Diplectrum formosum*), spottail pinfish (*Diplodus holbrooki*), red grouper (*Epinephelus morio*), rock hind (*Epinephelus adscensionis*), speckled hind (*Epinephelus drummondhayi*), butter hamlet (*Hypoplectrus unicolor*), gag grouper (*Mycteroperca microlepis*), pygmy sea bass (*Serraniculus pumilio*), tattler (*Serranus phoebe*), and belted sandfish (*Serranus subligarius*); lane 15, no DNA control.

PCR versus RFLP assays

The species-specific PCR and RFLP assays can be used to accurately identify larvae. The decision of which assay to use could be based on labor and reagent costs. The PCR assays are attractive because they are rapid single step assays, whereas the RFLP assays employ PCR in the first step and restriction enzyme digestion of the PCR products in the second step. In most cases, PCR reagents are more expensive than those needed for restriction enzyme analysis. However, PCR assays can be cost effective if they are multiplexed. Excellent examples of successful multiplex PCR assays are described by Chapman et al. (2003) and Hyde et al. (2005). Unfortunately our attempts at multiplexing the assays were unsuccessful because of the production of nonspecific PCR products.

RFLP assays are cost effective because they require less expensive reagents than PCR assays. However, the RFLP assays require an extra hour or two to process and involve additional labor costs. The RFLP assays developed here employed genus-specific primers rather than species-specific primers to amplify *Centropristis* genomic DNA from adult fish (Table 3). Using genus-specific primers, we simplified the PCR reaction mixtures for all three species and ensured that only *Centropristis* DNA would generate PCR products. The resulting PCR products were subsequently digested with *Alu* I enzyme to produce species-specific fragments that were easily distinguished by their unique banding patterns once electrophoretically separated on an agrose gel. The unique sizes of these fragments were due to species-specific differences in the locations of the *Alu* I restriction sites within the ITS1, 5.8S gene, and ITS2 regions (Fig. 6B).

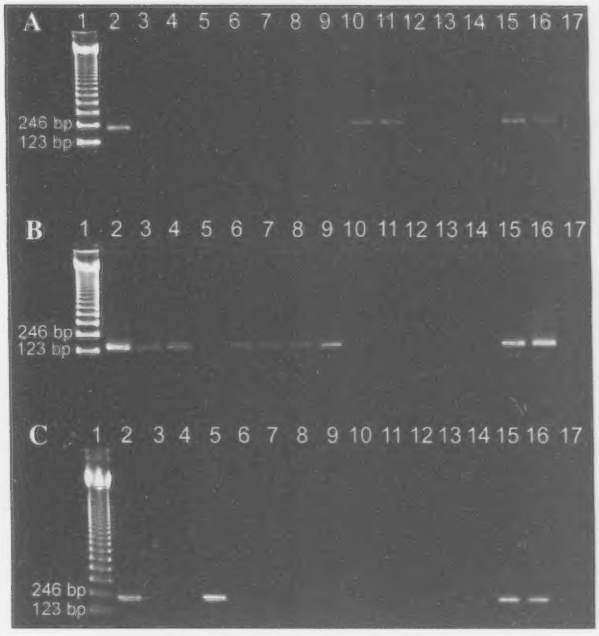

Figure 5

Results of species-specific polymerase chain reaction (PCR) assays for sea bass (*Centroprisitis*) larvae (**A**) black sea bass (*Centropristis striata*), (**B**) bank sea bass (*C. ocyurus*), and (**C**) rock sea bass (*C. philadelphica*). In each panel, the gels were loaded in the following manner: lane 1, 123-bp ladder; lane 2, positive controls for A (*C. striata*), B (*C. ocyurus*), and C (*C. philadelphica*); lanes 3–13, *Centropristis* larvae, lane 14, negative control; lane 15–16, PCR inhibition controls; lane 17, no DNA control.

Shipboard operational molecular assays

The PCR and RFLP assays could be conducted at sea with a small thermocycler and the other minor equipment necessary for DNA extraction. If onboard conditions were favorable, the assays could be analyzed immediately after PCR thermocycling by gel electrophoresis and with the use of a digital imaging camera. Similar methods described by Hyde et al., (2005) were used at sea to identify the eggs and larvae of blue marlin (*Makaira nigricans*), shortbill spearfish (*Tetrapturus angustirostris*), and wahoo (*Acanthocybium* solandri). Their procedures incorporated a boiling method to quickly prepare amplifiable DNA from fresh tissue. If this would not be practical, then PCR products could be frozen and analyzed at an onshore laboratory. Results for 30 larval assays could be attained in four hours or less at a cost of ~$5 per sample, excluding the cost of equipment. Alternatively, larvae could be collected and sorted; all *Centropristis* larvae would be separated and assayed or stored individually in 95% ethanol for processing at a shore-based laboratory. DNA could then be extracted from a small tissue sample (e.g., an eye) from each specimen. The intact larvae would be returned to storage in 95% ethanol in case any additional morphological or molecular evaluation was required. If analysis of a large number of samples was desired, the individual PCR assays could be adapted to a SYBR green quantitative PCR format. Because this approach eliminates the need for gel electrophoresis, it could be used to process hundreds of samples provided that sorting could be expedited.

Conclusion

Both the species-specific PCR and RFLP assays described in this article can successfully identify *C. striata*, *C. ocyurus*, and *C. philadelphica*. The assays are rapid, cost effective, simple to perform, and highly accurate.

Furthermore, these techniques can be integrated with environmental and oceanographic data to allow almost immediate investigation of spawning times, spawning locations, and larval dispersal. The ability to identify *Centropristis* larvae to species throughout their range will provide a foundation for future studies where early life history stages of fishes are used to investigate questions related to fisheries management. This study also provides a model for the future development of species-specific assays for other commercially important fish species.

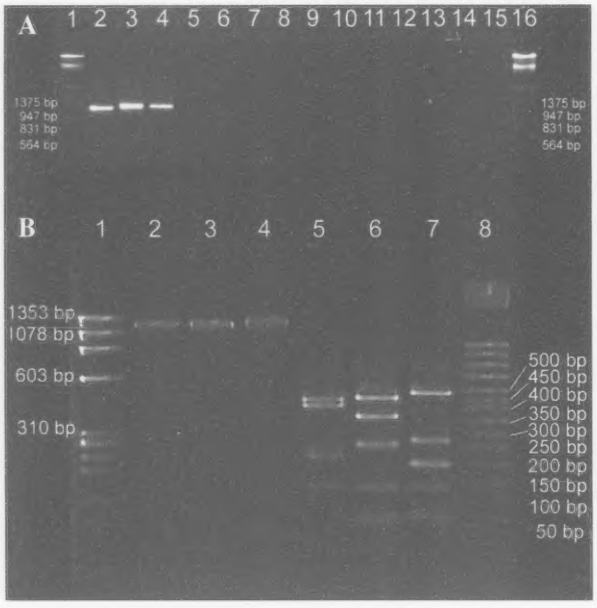

Figure 6

(**A**) Cross-reactivity test of genus-specific polymerase chain reaction (PCR) primers (CentropFW1 and CentropRev1) used for restriction fragment length polymorphism analysis (RFLP) of black sea bass (*Centropristis striata*), bank sea bass (*C. ocyurus*), and rock sea bass (*C. philadelphica*). The gel was loaded in the following order: lanes 1 and 16, DNA molecular weight marker III (Roche Diagnostics, Indianapolis, IN); lane 2, *C. striata*; lane 3, *C. ocyurus*; lane 4, *C. philadelphica*; lanes 5–14, sand perch (*Diplectrum formosum*), spottail pinfish (*Diplodus holbrooki*), red grouper (*Epinephelus morio*), rock hind (*Epinephelus adscensionis*), speckled hind (*Epinephelus drummondhayi*), butter hamlet (*Hypoplectrus unicolor*), gag grouper (*Mycteroperca microlepis*), pygmy sea bass (*Serraniculus pumilio*), tattler (*Serranus phoebe*), and belted sandfish (*Serranus subligarius*); lane 15, no DNA control. (**B**) PCR-RFLP analysis of *Centropristis* ITS1, 5.8S, and ITS2 rDNA amplified with genus-specific primers described in the text. Each PCR product was digested with the restriction enzyme *Alu* I. Lane 1, DNA molecular weight marker IX (Roche Diagnostics, Indianapolis, IN); lanes 2–4 PCR products for *C. striata*, *C. ocyurus*, and *C. philadelphica*; lanes 5–7, *Alu* I digests of PCR products for *C. striata*, *C. ocyurus*, and *C. philadelphica*; lane 8, 50-bp ladder.

Acknowledgments

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The vulnerability of reproductively active squaretail coralgrouper (*Plectropomus areolatus*) to fishing

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Abstract—Squaretail coralgrouper (*Plectropomus areolatus*) were captured and tagged at a fish spawning aggregation (FSA) site with conventional and acoustic tags to assess their vulnerability to fishing and spatial dynamics during reproductive periods. Males outnumbered females in catch and, on average, were larger than females. Findings revealed a high vulnerability to fishing, particularly during reproductive periods, and most fish were recaptured within the 5-month spawning season and within 10–12 km of the aggregation site. Individual and sex-specific variability in movement to, and residency times at, the FSA site indicates that individual monthly spawning aggregations represent subsets of the total reproductive population. Some individuals appeared to move along a common migratory corridor to reach the FSA site. Sex-specific behavioral differences, particularly longer residency times, appear to increase the vulnerability of reproductively active males to fishing, particularly within a FSA, which could reduce reproductive output. Both fishery-dependent and fishery-independent data indicate that only males were present within the first month of aggregation. The combined results indicate that reproductively active *P. areolatus* are highly vulnerable to fishing and that FSAs and migratory corridors of reproductively active fish should be incorporated into marine protected areas. The capture of *P. areolatus* during reproductive periods should be restricted as part of a comprehensive management strategy.

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Epinepheline serranids (groupers, hinds, lyretails) that form fish spawning aggregations (FSAs) are among the most vulnerable coral reef fishes to overexploitation (Coleman et al., 2000; Sadovy and Domeier, 2005). Indeed, 20 of 162 epinepheline serranids (hereafter, serranids), including species that form FSAs, are now considered vulnerable or endangered according to IUCN Red List criteria. Among those listed as vulnerable is the squaretail coralgrouper (*Plectropomus areolatus*) (Rüppell, 1830) that forms temporally and spatially predictable FSAs, often numbering in the 100s to 1000s of individuals. These traits contribute to the targeting of *P. areolatus* by local and foreign commercial fisheries, including the Southeast Asia-based live reef food fish fishery (Sadovy et al., 2003). When both FSAs and reproductively active individuals are targeted enroute to spawning sites, reproductive populations may experience population-level changes that affect reproductive output, including skewed aggregation sex ratios following sexual selection (e.g., Koenig et al., 1996), size reductions (e.g., Beets and Friedlander, 1998), changes in genetic diversity (Chapman et al., 1999), and loss or decline in FSA (e.g., Sadovy and Domeier, 2005). With few exceptions, FSA fishing is unsustainable under anything more than light fishing pressure, such as that characteristic of limited subsistence-level fishing (Sadovy and Domeier, 2005). Reports of grouper FSA loss continue to increase worldwide (e.g., Matos-Caraballo et al., 2006; Aguilar-Perera, 2007) and increased fishing mortality at FSAs is implicated in local fishery declines (Sadovy and Domeier, 2005). Among measures recently touted to protect FSAs and reproductive populations from overfishing are marine protected areas (MPAs). However, few reports show the effectiveness of MPAs in limiting or preventing FSA loss, or in improving population-level abundance (Nemeth, 2005). Moreover, most existing FSA-based MPAs are small and likely leave reproductively active individuals vulnerable to fishing, such as some reproductively active serranids that appear to use common migratory corridors to reach FSAs, where they can be fished before they spawn (Nemeth et al., 2007; Starr et al., 2007). Migratory corridors and the dangers of overexploitation when corridors are left unprotected are recorded for a number of fishes, such as gadids (e.g., Rose, 1993). To design effective management for reproductive populations of serranids such as squaretail coralgrouper, areas of high fishing vulnerability must be identified, including FSA sites, migratory corridors, and other areas where fish may concentrate during reproductive periods.

Within the tropical Pacific, only Palau and Pohnpei have developed long-term management protocols at the national level specifically to protect

commercially important serranids (*P. areolatus*, *Epinephelus fuscoguttatus* [brown-marbled grouper], and *E. polyphekadion* [camouflage grouper]) during reproductive periods (Rhodes and Sadovy, 2002). Temporary community-based MPAs are used elsewhere, such as at the Solomon Islands and Papua New Guinea, whereby fishing is allowed at FSAs after periods of build-up within the aggregating population. For Pohnpei, where the current study was focused, management protocols include a small-scale MPA that protects *P. areolatus*, *E. polyphekadion*, and *E. fuscoguttatus* at a common FSA site (Kehpara Marine Sanctuary [KMS]) and a seasonal serranid sales ban that coincides with peak reproductive periods for these species (March–April). However, these measures fall short of fully protecting these and other FSA-forming serranids because 1) some unprotected FSAs continue to be fished (e.g., at nearby Ant Atoll and Palikir Pass); 2) sales bans do not cover entire spawning seasons for the aforementioned species; and 3) some serranids appear to reproduce outside the sales ban period (Rhodes and Tupper, 2007). Migratory corridors have not been investigated locally, including around the KMS, and thus reproductively active individuals possibly exist outside current MPA boundaries and are, therefore, vulnerable to exploitation. Finally, subsistence catch for aggregating serranids, shown to lead to overexploitation elsewhere (e.g., Olsen and LaPlace, 1979), remains open year round, including during the sales ban period.

In Pohnpei, *P. areolatus* is a favored target of local subsistence fishermen and small-scale commercial fishermen, representing approximately 12% of the combined-gear commercial coral reef grouper fishery that includes 24 species (Rhodes and Tupper, 2007). The species is taken year-round largely as juveniles and small adults from inner reef areas during nighttime spearfishing activities, but is also known to be targeted at FSA sites, including at least three known unprotected FSA sites. Anecdotal evidence indicates that one local *P. areolatus* FSA may have been extirpated in the 1990s after continued targeting of an annual reproductive migration that ceased to form around 1995. Gravid *P. areolatus* are commonly found in markets during reproductive months outside the March–April serranid sales ban period, occasionally in the hundreds of individuals, and there is concern that reproductively active *P. areolatus* from the KMS may be targeted along migratory corridors or other areas where fish congregate (i.e., staging areas) for noncommercial use during sales-ban periods.

Using both conventional and acoustic tag-recapture techniques, we examined the vulnerability of squaretail coralgrouper to fishing by examining spatial and temporal trends of tagged individuals at a single protected spawning aggregation site. The study objectives were 1) to determine potential sexual differences in behavior (within reproductive periods) that may facilitate capture and impact reproduction; 2) to examine the potential catchment area(s) (determined from recaptures) from which reproductive populations of *P. areolatus* at KMS may be drawn; 3) to assess the direction and distance of movement of fish in relation to the FSA to improve MPA design; 4) to identify potential migratory corridors of spawners in relation to KMS where fishermen may concentrate fishing activities; 5) to determine if the existing MPA at KMS adequately protects reproductively active individuals; and 6) to assess whether current fisheries management could be improved to reduce the capture of reproductive fishes during spawning periods, particularly at the FSA site. Estimates of overall fishing mortality and fisheries sustainability for squaretail coralgrouper in Pohnpei are outside the scope of the current study because information on stock characteristics and the fishery (particularly subsistence) remain incomplete and, therefore, statements concerning these indices would be premature.

Material and methods

Site description

The tagging study was conducted at the Kehpara Marine Sanctuary, a known protected FSA site in Pohnpei, Federated States of Micronesia (6°55′N, 158°15′E), where *P. areolatus*, *E. polyphekadion*, and *E. fuscoguttatus* aggregate seasonally to spawn (Fig. 1). *Plectropomus areolatus* forms aggregations annually from ca. January–May at KMS, seaward of the barrier reef along approximately 0.5 km of the reef flat and wall at ~10–30 m depth (first author, unpubl. data). During the aggregation period, individuals seek shelter and remain in relatively close proximity (<5 m) to the low-relief coral that covers much of the site. During daytime, individuals can be observed meandering among large high-relief stands of coral in shallower portions of the reef flat and over the intermittent sand patches and rubble piles that are found throughout the area. Although *P. areolatus* aggregations have been monitored at the site over a 7-yr period, spawning has never been observed within aggregation months. Histological analyses of gonads have recently confirmed a seasonal and lunar periodicity of spawning for this species (first author, unpubl. data).

Fish capture, catch per unit of effort, and tagging

To capture fish for tagging, two locally hired fishermen used live bait (*Myripristis* sp.), hook-and-line, and mask and snorkel to target *P. areolatus* from the surface within the FSA site. Fishing was conducted daily over 5 days in January and 7 days monthly from February through May just before and including the full moon, for a total of 33 fishing days. Target depths for fishing ranged from ~15–30 m where fish aggregate within the FSA site. To estimate catch-per-unit-of-effort (CPUE), daily soak times and catch volumes were recorded from February to May. No CPUE estimates were made in January, although fishing methods and times were similar among months. Following capture and before processing, fish were brought onboard and anesthetized in a 0.75 g/L

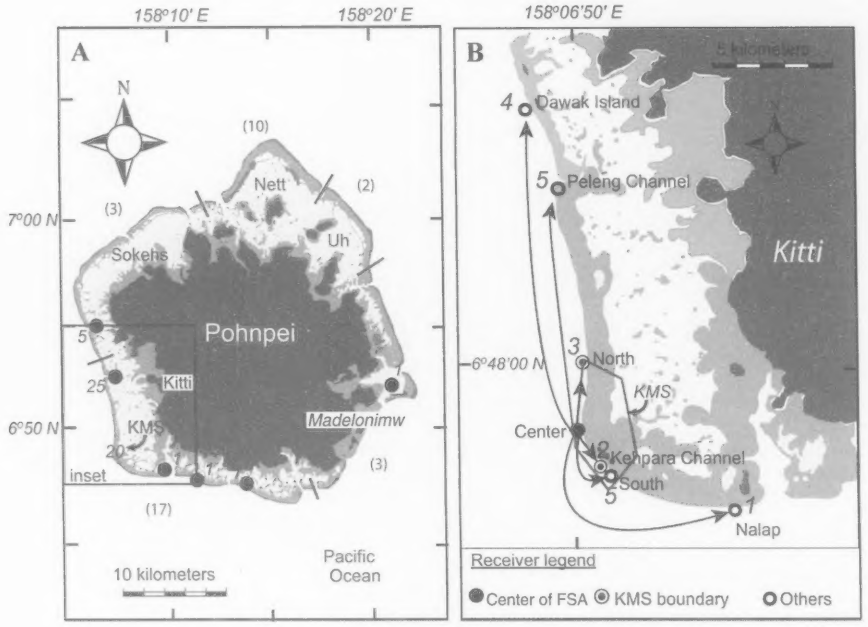

Figure 1

(**A**) Map of the Pohnpei Island showing the general catch locales (circles) and number (italics) of recaptured conventionally tagged squaretail coralgrouper (*Plectropomus areolatus*). Catch locales are (counterclockwise from left) Dawak, Peleng Channel, Nalap, Liap, Penieu, and Temmen. Catch probabilities (in parentheses), or the number of recaptures anticipated within a municipal reef area, are based on the total number of recaptures from this study (January 2005–February 2006) divided by the percent fishing effort allocated to a municipal reef area. Effort estimates were derived from a 2006 market survey that included interviews of 1123 commercial reef fishermen (Rhodes et al., 2007). Estimates of catch probability exclude recaptures with unreported catch locales (n=5) and fish captured by researchers inside the Kehpara Marine Sanctuary (KMS) (n=20). Municipalities (Nett, Sokehs, Kitti, Madelonimw, Uh) are separated by black diagonal lines. (**B**) Inset map shows the direction of movement and number of individuals detected by Vemco VR2 acoustic receivers outside the KMS during the study period. The Kehpara Marine Sanctuary is represented by the area outlined in gray. FSA=fish spawning aggregation. Others=other receivers.

tricane methanesulfonate-seawater solution until fish lost equilibrium (~3–5 minutes). Following anesthesia and air bladder deflation, all individuals were weighed (nearest g body weight), measured (nearest mm total length [TL] and standard length [SL]), and sex was determined macroscopically by using a 1-mm bore nylon cannula (Rhodes and Sadovy, 2002).

To determine the potential distance of movement, times at liberty and catchment areas (defined as the area from which spawning individuals are drawn [Sadovy and Domeier, 2005]), all captured fish were tagged with a uniquely numbered Floy FT-1-94 conventional tag (Floy Tag, Seattle, WA) that provided contact and reward information. Of these Floy-tagged specimens, 40 fish (20 males and 20 females) were surgically implanted with Vemco V16 acoustic transmitters (Vemco AMIRIX Systems, Halifax, Nova Scotia) in January and February. For tag implantation, abdominal incisions (~3.5 cm) were made just anterior to the vent and were closed with ConMed Reflex One® 35 Wide surgical skin staples (ConMed Endosurgery, Utica, NY) (Tupper and Able, 2000). After surgery, fish were allowed to recover 10–20 min onboard in fresh aerated seawater before release into shallow (2–5 m) water near the reef crest. Most acoustic-tagged and some Floy-tagged fish were observed from the surface by snorkel divers to follow initial recovery and to monitor potential predation.

Acoustic tracking and tag recovery

For acoustic tracking of fish distance and direction of movement, and to determine residency times within the FSA, a total of 7 Vemco VR2 receivers were moored in January 2005 at the following locations: center of the FSA, at north and south KMS boundaries, within the Kehpara and Peleng channels adjacent to the FSA, and seaward of Dawak and Nalap islands that are north and south, respectively, of channels adjacent to the FSA

Table 1

Summary table of sex-specific catch of squaretail coralgrouper (*Plectropomus areolatus*) at the aggregation site during tagging in 2005. *N* = total number of individuals collected during the reproductive season; *n*=monthly sample size; FDC = first day of catch before the full moon; na = data not available.

		Month									
		January		February		March		April		May	
Sex	*n*	FDC	*n*	FDC	*n*	FDC	*n*	FDC	*n*	FDC	*N*
Male	71	4	125	4	96	6	130	9	89	8	511
Female	0	na	32	3	38	5	37	5	22	5	129
Unknown	4	3	0	na	0	na	0	na	3	8	7
Total	75		157		134		167		114		647

(Fig. 1). All receivers were tethered above the barrier reef at 10–25 m depth at seaward-facing promontories to maximize detection range. All receivers were removed after 17 months that covered two full consecutive spawning seasons. Prior range testing in Palau under similar physical and oceanographic conditions was performed for 13 receivers along four cardinal directions with a line-attached transmitter. The transmitter was towed behind the boat and away from the receivers, and times and distances from the receiver were recorded by GPS. Distance and times were later matched to receiver-logged transmissions to determine minimum and maximum detection distances. Testing confirmed an average maximum detection distance of 442 m along the reef parallel to the barrier reef crest and 975 m from the receiver seaward. Within the channel (here, Ulong Channel), the receiver detected tags at an average of 746 m parallel to the channel, while distance perpendicular to the channel was constrained by the channel width, similar to the constraints for detection distances from the receiver to the reef crest (perpendicular to the barrier reef).

To enhance the potential for tag recovery, a reward scheme (US$5 plus fish market value) was broadcast island-wide on local AM radio and by flyer postings. Records for catch location and gear type were taken from local fishermen at the time of reward. Recaptured fish were reweighed and measured, and gonads and otoliths were extracted for subsequent life history analyses.

Analysis of catch probability

A separate creel survey was conducted in 2006 to determine the distribution of fishing effort within Pohnpei's small-scale commercial coral reef fishery (Rhodes et al., 2007). The 2006 effort data were used to estimate the number of likely recaptures for *P. areolatus* under a random distribution (postspawning dispersal) scenario (chi-square analysis, after a square-root transformation) for each municipality. For this analysis, those fish captured by researchers inside the KMS were excluded, as were an additional five individuals for which there was no recorded recapture location.

Results

Initial capture numbers, CPUE, and size distribution of fish

Between January and May 2005, 647 *P. areolatus* were captured and tagged at the Kehpara Marine Sanctuary over 33 fishing days and 170 fishing hours, with an estimated CPUE of 3.8 fish per hour per fisherman, to highlight the vulnerability of reproductive *P. areolatus* to hook-and-line fishing at the aggregation site. Catch included 511 males, 129 females, and 7 individuals that could not be identified to sex with macroscopic methods. Males typically preceded females in catch by 1–4 days, except in January when no females were taken (Table 1). The average sex ratio for catch (February–May) was 3.4 males:1 female, indicating either an absence of females within the January FSA or highly variable sex-specific differences in catch between January and other months. Late-stage vitellogenic oocytes were observed in all cannulated females, and no females were captured with hydrated oocytes (to indicate imminent spawning), although subsequent histological examination of gonads from recaptured individuals confirmed evidence of spawning activity. All males were ripe at the time of capture during all months of tagging.

Males captured at the FSA were, on average, larger than females. The size frequency range was 378–542 mm TL for mature females (mean ±standard error [SE]=458.0 ±2.8 mm TL], and 450–660 mm TL for mature males (mean=541.4 ±1.3 mm TL) (Fig. 2). Only males were present in size classes above 550 mm TL, and only females were observed in size classes below 440 mm TL. Individuals of undetermined sex ranged from 480 to 575 mm TL (mean=523.3 ±14.6 mm TL) and thus overlapped in size with mature males. The mean size of captured males declined slightly during the survey, while captured female mean size increased. During tagging, similar behavior, including courtship and territorial displays, was observed between tagged and untagged fish to indicate minimal or no effects from tagging on at least some behavior commonly associated with reproduction.

Conventional tagging

The majority of conventional tag recaptures occurred within the January–May spawning season and thus indicated that *P. areolatus* are most vulnerable during reproductive periods, whereas the potential for sexual selection to reduce reproductive output was reflected in the male-dominated capture and recapture sex ratio and the high spawning-site fidelity shown by males within the reproductive season. Of the 647 *P. areolatus* tagged, 59 individuals (9.1% of the total) were recaptured (Table 2), including 39 individuals recaptured by commercial fishermen and 20 individuals recaptured at the KMS by researchers during tagging. All individuals recaptured at the KMS were males. Among the males recaptured at KMS, 50% were taken one month after tagging, 15% were taken after two months, 20% after three months, and 5% after 4 months. At the KMS, one male was recaptured twice in the same month and another male was taken three times over three separate months—a finding that demonstrates that at least some individuals return repeatedly within the spawning season. Outside the KMS, fishermen recaptured 27 males, eight females, and one fish of unknown sex (at the time of tagging). Three additional recaptures by the fishery could not be identified either to sex (owing to illegibility of the information on the tag) or to recapture location (which was unreported) or both sex and recapture location were not identified. The fishery recapture sex ratio (individuals of known sex taken outside the KMS) was 3.4:1 male:female, which matched the sex ratio of fish captured during initial tagging within the KMS (Table 1). Recaptured fish remained at liberty from 1 to 328 days, averaging 71 days for those taken by the fishery and 51 days for fish retaken within the KMS by researchers. The maximum straight-line distance traveled by a recaptured fish was ~27 km between the KMS and Temmen (Fig. 1A).

Squaretail coralgrouper appear highly vulnerable to fishing within and in areas immediately near the FSA site. A total of 97.1% of all fish were recaptured within 12 km of the FSA site during the 17-month survey period, and 88% of the recaptures with reported locations were taken from areas north of the FSA. The fishery made 61.5% of all recaptures within the 5-month spawning season, and 25.6% of all recaptures occurred during the March–April sales ban period, which demonstrates the ineffectiveness of the ban in protecting reproductively active fish (Table 2). Reported recaptures by the fishery were primarily recaptured fish from the inner reef, including a substantial number from areas immediately adjacent to Peleng Channel (42% of all individuals) that included 88% of all females and 64% of male recaptured. The distribution of fishery recaptures significantly deviated from expectations (χ^2=10.911, $0.25<P<0.05$) based on known catch records and area-specific effort data taken from 2006 creel surveys of the commercial reef fish fishery (Fig. 1). Only 3 of 59 recaptured fish were from locations south of KMS (Liap, Nalap, and Penieu), and only one individual was recaptured from the eastern side of the island (Fig. 1).

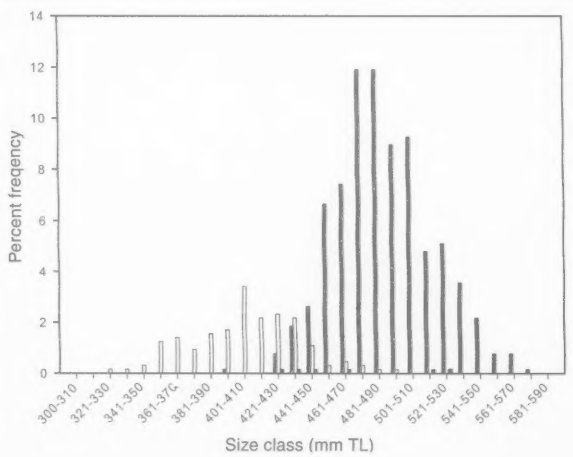

Figure 2

Size frequency distribution (mm total length, TL) of squaretail coralgrouper (*Plectropomus areolatus*, n=647) taken by hook and line from the Kehpara Marine Sanctuary during the initial tagging exercise in 2005. White bars=females; black bars=males.

Acoustic tagging

Of the 40 acoustically tagged fish, three individuals (7.5%) were recaptured by the fishery (January 2005: 2 males; March 2005: 1 female). Immediately after recapture, three replacement tags were deployed (February 2005: 2 males; April 2005: 1 female). Of the 43 total tag deployments, signals following initial detections were received from 13 of 22 males and 10 of 21 females (54% overall) to indicate high tag-induced mortality immediately after tagging or a rapid post-tag departure of individuals from the FSA without subsequent return. No predation was observed at KMS during snorkel or dive operations after the release of tagged individuals.

For those acoustically tagged individuals returning to the FSA, patterns of movement (seasonal and annual site visitation) were highly variable both within and among individuals (Table 3), although some individuals appeared to move along a common migratory corridor to reach the FSA where fishermen could potentially concentrate efforts and substantially impact reproductive populations. Data from four females and eight males provided point-to-point directional data to show a predominantly northward movement away from the FSA (i.e., north of the KMS southern MPA boundary) after spawning (Fig. 1B)— a movement that is reinforced by recapture data from conventional tagging (Fig. 1A).

Table 2

Temporal distribution of recaptured squaretail coralgrouper (*Plectropomus areolatus*) taken outside (by the fishery) and inside (by researchers) the Kehpara Marine Sanctuary (KMS). The entire shaded area represents the reproductive season and the sales ban period is darkly shaded. Months are abbreviated. CP, total = cumulative percent of total catch by month; CP, ban = cumulative percent contribution of the total recaptures within the sales ban period, including those by fishermen and researchers. All = the combined recaptures by the fishery (outside the KMS) and by researchers (within the KMS); *n* = sample size of recaptures within the study period; CP sample size (*n*) is that used to calculate cumulative percents; a = Peleng Channel or proximity; b = Dawak Channel or proximity; c = Nalap or vicinity; d = unknown locale; e = Liap; f = Penieu; g = Temmen.

		Year														
		2005											2006			
		Months											Months			
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	*n*
Fishery	Recaptures	1[a]	5[a,d]	7[a,c]	3[a,b]	8[a,b,c]	3[a,b]	0	0	1[e]	1[a]	6[a,d,f]	0	2[a,g]	2[a]	39
	CP, total	3	15	33	41	62	69	69	69	72	74	90	90	95	100	39
	CP, ban	—	—	18	26	—										10
KMS	Recaptures	0	2	6	5	7										20
	CP, total	0	10	40	65	100										20
	CP, ban	—	—	30	55	—										11
All	CP, total	2	14	34	49	75	80	80	80	81	83	93	93	97	100	59
	CP, ban	—	—	22	36	—										21

Table 3

Presence (●) of acoustically tagged squaretail coralgrouper (*Plectropomus areolatus*) to show the observed variability in residency times (as site visitations) at the Kehpara Marine Sanctuary fish spawning aggregation (FSA) site over a 17-month period (January 2005–May 2006). The study period encompasses two complete reproductive seasons (~January–May). The table represents only individuals present at the FSA for one month or more after initial tagging.

	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
Males	●	●	●	●	●												
	●	●															
	●	●	●														
	●	●	●												●	●	
	●	●	●														
	●	●	●	●	●	●	●	●	●	●		●	●		●	●	
	●	●	●	●													
	●	●		●	●	●	●	●	●	●	●						
Females		●	●		●	●	●						●	●	●	●	●
		●	●														
		●				●											
				●													
		●	●	●				●									

Travel speed for three fish that moved between Peleng Channel and the KMS (=8 km) was 2.1 km/h (1 female, 2 males), and all fish made the trip in ≤1 day. Although several acoustically tagged individuals moved southward to the KMS boundary, only one appeared to travel as far as Nalap. The apparent limited movement southward from the KMS is also reflected in the low number of conventional tag recaptures (n=4) from areas south or east of KMS. No movement was detected directly within Kehpara Channel, in contrast to previous anecdotal reports of post-spawning migrations through the channel. Some individuals were detected seaward of the channel.

Residence times at the FSA were sex-specific, thus creating a potential to promote sexual selection and negatively impact reproductive output when fishing was concentrated on the FSA. From acoustic results, we determined that males remained at the KMS over longer periods both within the reproductive season (2.2± 0.3 SE mo., males; 1.6 ±0.2 mo., females) and during the 17-mo. survey period (3.0 ±0.7 mo., males; 1.9 ±0.4 mo., females) (Table 3). Conversely, according to the acoustic data, males and females appeared to overlap entirely within a reproductive month. This overlap was not reflected in fisheries-dependent data because no females were captured ≥5 days before a full moon, whereas males were taken as early as 8–9 days before a full moon, indicating sex-specific variations in feeding or catchability.

Virtually all males acoustically tagged in January remained at the aggregation site until spawning was concluded in February, further enhancing the potential for sexual selection before actual spawning within the aggregation period. Few females appeared to remain at the FSA site after spawning during subsequent months. Only three individuals were present in all possible spawning months, and two males and three females returned to or resided at the KMS during nonreproductive periods (Table 3).

Discussion

The vulnerability of FSAs to overfishing is widely recognized (Sadovy and Domeier, 2005) and the present study shows the conditions under which FSA loss and possible population decline can occur. For example, under a hyperstable fishing scenario (no CPUE reduction in relation to abundance) and using CPUE estimates from this study (3.83 fish per hr per fisherman) and peak FSA abundance estimates for squaretail coralgrouper at KMS, only 250 fishing days at 6 h of fishing per day would be required to deplete the entire FSA, equivalent to only 36 fishermen fishing over 7 days. Such a scenario was borne out in 1998 when an estimated 4000 reproductively active fish were taken from the adjacent camouflage grouper FSA in just over 7 days (Rhodes and Sadovy, 2002). During that event, up to 20 boats with 2–3 fishermen/boat fished the aggregation daily with no observed change in CPUE, before the aggregation was included into the KMS by executive order in 1999. Underwater visual census (UVC) counts in later years showed that only several hundred camouflage grouper remained at the camouflage grouper FSA site and that only minor increases in abundance were apparent after 7 years of monitoring (2001–07). Similarly heavy aggregation fishing has resulted in either loss, near-extirpation, or a substantial reduction in FSA abundance elsewhere (e.g., Craig, 1969; Sadovy and Eklund, 1999; Sala et al., 2001).

Migratory corridors used by reproductive fish increase the vulnerability of *P. areolatus* (and other fishes) to overfishing by concentrating reproductively active or resting fish within confined areas similar to the FSA in our study. Although it is not clear whether all *P. areolatus* (at KMS or elsewhere) use these migratory corridors, several small groups of squaretail coralgrouper (5–10 individuals, presumably males) were observed moving southward along the reef between Peleng Channel and KMS and groups of up to 100 females (identification based on size and color) were observed moving in deeper water (20–30 m) toward the FSA during the spawning season. Groups of roving female squaretail coralgrouper have also been reported near FSA in the Solomon Islands during reproductive periods. Other serranids reported or suggested to use migratory corridors to reach FSAs include *Epinephelus guttatus* (red hind) in the U.S. Virgin Islands (Nemeth, 2005), *P. areolatus* in Palau, and *Epinephelus striatus* (Nassau grouper) in Belize (Starr et al., 2007). In each case, fishermen apparently target the unprotected areas where fish congregate, thereby reducing the effectiveness of existing MPAs to protect reproductive individuals, as observed in Pohnpei. The apparent targeting of these migratory corridors and the ease by which fishermen can remove individuals from these and other areas where fish congregate highlights the need to identify and incorporate those areas into MPAs.

The observed sex-specific differences in the behavior of *P. areolatus* within the reproductive season have the potential to promote selective fishing and to impact the spawning sex ratio and reproductive output (e.g., Beets and Friedlander, 1998). In Pohnpei, sex-specific behavioral differences were manifested as 1) a greater abundance of males in FSA-catch both seasonal and monthly, particularly in January when no females were captured; 2) the persistence of males at the FSA over several weeks during the initial aggregation period (January–February); and 3) longer male residency times at the FSA seasonally. Similar to the current study, previous studies of FSA-forming serranids have also shown that males often precede females to the FSA and, therefore, have longer residency times (Samoilys, 1997; Rhodes and Sadovy, 2002; Starr et al., 2007). For squaretail coralgrouper, longer residency times appear to increase the catchability of males. Alternatively, males may outnumber females at the FSA site in all months and be reflected in the catch sex ratio. Further fisheries-independent assessments of aggregation (and population) sex ratio are needed to confirm whether males indeed outnumber females. Acoustic data also indicate that in addition to taking bait over more days than females, male squaretail coralgrouper frequent the FSA over more months—a finding that is similar to those from acoustic surveys of Nassau grouper in Belize (Starr et al., 2007) and UVC monitoring of freeze-brand tagged leopard coralgrouper (*P. leopardus*) along the Great Barrier Reef (Zeller, 1998). Fisheries-dependent size data from FSA catch have also indicated protracted residency times for male red hind in the U.S. Virgin Islands (Nemeth, 2005). Protracted male residency time may increase the potential for sexual selection, leading to adverse affects on reproduction, such as sperm limi-

tation (Coleman et al., 1999). Similarly, the removal of larger individuals (males in protogynous species) may lead tc reductions in the mean size of both males and females (from compensated sex change) to reduce overall fecundity (Vincent and Sadovy, 1998) and, in the case of extreme operational sex ratios, may adversely affect reproductive behavior. At the KMS FSA, males appeared to be more vulnerable to line fishing than were females, comprising 79% of the catch during tagging operations and representing all recaptures within the KMS. Males also dominated recaptures by the fishery among spear-caught individuals. Interestingly, females and juvenile squaretail coralgrouper dominated marketed catch of squaretail coralgrouper overall, and 97% of the marketed catch in 2006 was smaller than the mean size of sexually mature males (Rhodes and Tupper, 2007). Thus, in Pohnpei, the potential currently exists for both growth overfishing and recruitment overfishing and there is an urgent need for a thorough examination of island-wide stock levels and sustainability within the squaretail coralgrouper fishery, as well as the sustainability of both the subsistence and other fisheries targeting FSAs. One possible explanation for the paucity of females in catch is that recaptured females went unreported or that fishermen targeted larger individuals (i.e., males) to benefit from the combined higher fish value and reward. An improvement to our study would be to perform real-time observations of the fishery at heavily fished locations, such as Peleng Channel, to verify the sex ratio of the catch.

Among acoustically tagged individuals, the data showed a high degree of variation in the seasonal pattern and duration of stay for individuals that frequent the FSA site. Although these results could be explained in part by mortality (natural, fishing, and tag-induced, particularly acoustic) or a failure of receivers to detect all fish present, similar variability has been shown elsewhere for serranids with the use of these and other tagging methods (e.g., Zeller, 1998; Starr et al., 2007). In Pohnpei, a gradual reduction in the number of acoustically tagged individuals returning to the FSA was observed within a reproductive year; only 50% of tagged individuals returned in the month after initial tagging. Similar findings were found for acoustically tagged Nassau grouper in Belize, where no more than 50% of tagged *E. striatus* males and 52% of tagged females were present during any reproductive month following tagging (Starr et al., 2007). Nassau grouper returning to the FSA after having been tagged showed a similar sequential decrease in numbers. On the Great Barrier Reef, only 31% of tagged mature leopard coralgrouper participated in spawning aggregation activities within a reproductive year; this percentage may indicate that individuals do not spawn annually (Zeller, 1998). Although Zeller (1998) suggested that tagged individuals may reproduce outside FSAs, spawning outside aggregations for FSA-forming species has not been shown. Likewise, high site fidelity has been shown for a number of serranids, with individuals observed moving past an active conspecific FSA to return to the site where they previously spawned. Sequential reductions in the number of conventionally tagged red hind have also been shown in the U.S. Virgin Islands (Nemeth et al., 2007). Although it is possible that the observed findings could be explained by natural or fisheries-related mortality, it seems more likely that some adults do not participate in all potential spawning months within the reproductive season and that some may not spawn in consecutive years. Although more investigations are needed, if these implications are correct, individual (monthly) FSAs represent subsets of the reproductive population, and these subsets complicate monitoring programs that solely use FSAs to estimate reproductive population abundance and changes therein. Regardless, these results indicate that the relationship between a FSA and the adult population is complex and additional work is needed on serranid reproductive dynamics for designing effective monitoring and management strategies for adult populations and in understanding impacts on these populations through FSA-targeted fishing.

The assessment of catchment areas for aggregating serranids by using tagging methods is relatively new, yet necessary to complete our understanding of reproductive population dynamics and to determine sustainability when fishing efforts are unevenly distributed. In Pohnpei, support for a catchment area of ~200–300 km^2 for the KMS-based squaretail coralgrouper FSA is provided from recapture data that indicates short-scale (10–12 km) movement of individuals in relation to the FSA after spawning. The observed recapture patterns do not appear to be strictly tied to area-specific fishing effort because Kitti municipality receives only 50% of fishing efforts statewide, yet >95% of recaptures occurred within a relatively small area of the municipality (Rhodes et al., 2007). Although it is possible that fishing effort during spawning months (and full moon periods) is more concentrated in Kitti than at other municipalities, such a pattern was not borne out from over 1000 interviews (2006) of fishermen participating in commercial fishing activities. Thus, for squaretail coralgrouper in Pohnpei, the reproductive population in relation to the KMS spawning aggregation appears to be highly localized and thus management could be applied to individual aggregations, in addition to combined aggregations within Pohnpei. Similar to sex-specific residency patterns, other studies of FSA-forming serranids also indicate that adults are concentrated within relatively small areas in relation to their respective FSA. For example, evidence exists for confined catchment areas for red hind determined from two FSAs in the U.S. Virgin Islands: 1) 500 km^2 for the St. Thomas FSA and 2) 90 km^2 for the St. Croix site FSA (Nemeth et al., 2007). The catchment area for Nassau grouper at the Glover's Reef Atoll FSA (Belize) appears to be no more than 384 km^2 (Starr et al., 2007). For *P. leopardus* along the Great Barrier Reef, Zeller (1998) estimated a catchment (migration) area of ca. 80 km^2. For these species, data clearly indicate limited movement by (most) individuals after spawning and highlight the potential for localized extinction and associated impacts to fish

community structure and fisheries from FSA fishing (or unsustainable fishing of individuals at earlier life history stages). The data also provide additional support to suggest that, while possible, long distance movement by reproductively active serranids may be more rare than previously assumed and that many aggregating grouper are resident to areas near the spawning site.

The effectiveness of the no-take year-round KMS to protect *P. areolatus* FSAs is evident, because without this protection, thousands of groupers could be removed within the spawning season and there would be the potential for rapid FSA loss and localized population extinctions. Regardless of the effectiveness of the KMS in protecting spawners at the spawning site, the potential for the fishery to impact the reproductive individuals still exists. Spawners are being taken along migratory corridors or other areas where fish congregate outside the KMS, with the potential to meet or exceed the level of fishing mortality experienced under aggregation fishing. In Pohnpei, problems with the existing sales-ban strategy are now evident. Firstly, 36% of all recaptured fish are taken during serranid sales ban months. Secondly, the serranid sales ban was recently shown to place added pressure on other equally vulnerable commercial species not currently managed, such as scarids (Rhodes et al., 2007). The increase in catch volume of scarids, for example, implies that fishermen have greater concern for maintaining catch volume than for the survival of individual species and indicates that efforts should be concentrated on measures to reduce overall catch volume. Similar reductions may be needed in catch volume of juveniles and small adults to stem potential recruitment overfishing. Thus, effective protection of reproductively active serranids will require innovative solutions that provide protection for both reproductive and nonreproductive individuals, while alleviating the potential negative effects of individual species management. The results from this and related studies highlight the complexities of reproductive population dynamics for aggregating serranids and support total protection through no-take bans during reproductive periods and no-take areas that support aggregations and associated areas where reproductive individuals congregate. Future studies should be designed to fill the gaps in our current understanding of serranid stocks in Pohnpei to enable a determination of sustainable harvest levels. Numerous examples globally indicate that the current harvest of reproductive adults and targeting of juveniles is currently unsustainable and that population declines and aggregation losses are imminent without a rapid move toward improved management.

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Mitochondrial DNA markers to identify commercial spiny lobster species (*Panulirus* spp.) from the Pacific coast of Mexico: an application on phyllosoma larvae

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Molecular markers based on mitochondrial DNA (mtDNA) are extensively used to study genetic relationships. mtDNA has been used in phylogenetic studies to understand the evolutionary history of species because it is maternally inherited and is not subject to genetic recombination (Gyllensten et al., 1991). The high mutation rate of mtDNA makes it a useful tool for differentiating between closely related species (Brown et al., 1979)—a tool that is especially important when significant variations occur between species, but not within species (Hill et al., 2001; Blair et al., 2006; Chow et al., 2006a).

Species-level identification, based on molecular markers, can be very useful when morphological features alone do not provide sufficient differentiation or when only part of an organism is recovered. In fact, a few authors have successfully applied genetic markers for species identification based on remains recovered from fecal samples, parts of specimens, processed products, or larval and juvenile forms (Chan et al., 2003; Purcell et al., 2004; Hsieh et al., 2007).

Three commercial lobster species inhabit the Pacific coast of Mexico: California spiny lobster (*Panulirus interruptus*, west coast of California and the Baja California Peninsula); blue spiny lobster (*P. inflatus*, southern Baja California Peninsula to the State of Oaxaca, Mexico); and green spiny lobster (*P. gracilis*, a tropical species from southern Baja California Peninsula to Peru) (Hendrickx, 1995). Taxonomic identification of adult lobsters is easily done by morphological features (Hendrickx, 1995); however, alternative techniques are required for identification of larvae when morphological features are unable to provide the means of identifying early life stages of spiny lobster species (Johnson, 1971; Muñoz-García et al., 2004). Furthermore, discrimination between larvae of *P. inflatus* and *P. gracilis* could be especially difficult because of their overlapping distribution.

The species-specific identification of larvae of other *Panulirus* species has also been difficult. Chow et al. (2006b) found intraspecific and intra-individual variation in appendage structures (the subexopodal spines) in the phyllosoma of *P. ornatus* (ornate rock lobster) and *P. versicolor* (painted spiny lobster). Therefore, subexopodal spine arrangements may not be a useful diagnostic for distinguishing between these two species. Because of these potential problems, several authors have suggested the use of molecular markers to identify spiny lobster larvae (Silberman and Walsh, 1992; Chow et al., 2006a, 2006b; Konishi et al., 2006).

In this study, the nucleotide variations of the mitochondrial DNA (mtDNA) in adult lobsters were investigated to obtain genetic markers useful in identifying *P. interruptus*, *P. inflatus*, and *P. gracilis* through either the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis or species-specific primers that amplify different size fragments in a multiplex reaction. These techniques were used to identify phyllosoma larvae collected outside the Gulf of California.

Materials and methods

PCR-RFLP analysis

DNA from adult specimens of the three species was taken from the following sites on the west coast of Mexico: *P. interruptus* from Baja California (n=2) and Baja California Sur (n=2); *P. inflatus* from Baja California Sur (n=3), Sinaloa (n=4), Nayarit (n=2), and Jalisco (n=1); and *P. gracilis* from Baja California Sur (n=2), Sinaloa (n=3), and Nayarit (n=1) (see Table 1).

A fragment of the 16S rRNA gene was amplified with primers 16Sar-L (5′-CGCCTGTTTATCAAAAACAT) and 16Sbr-H (5′-CCGGTCTGAACTCAGATCACGT) (Palumbi, 1996).

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Table 1

Sampling sites and dates for each lobster species along the Pacific coast of California and Mexico. The number of samples in the Seq1-16S column refers to the sequences used to find diagnostic restriction sites to discriminate lobster species; RFLP and Multiplex columns are the number of larvae analyzed by the RFLP and multiplex analysis, respectively; Seq2-16S column are the sequences used to evaluate consistency of restriction patterns.

			Number of samples			
Sampling sites	Location	Sampling date	Seq1-16S	RFLP 16S	Multiplex 12S-CR	Seq2-16S
Panulirus interruptus (California spiny lobster)						
Catalina Island, CA, USA	33°26′, 118°29′	Mar 2003			1	
Ensenada, B.C., Mexico	31°50′, 116°38′	Aug 1999	1	3	1	
Isla Guadalupe, B.C., Mexico	29°00′, 118°10′	Dec 2002	1	3	1	
Punta Eugenia, B.C.S., Mexico	27°49′, 115°06′	Jun 1999		2	1	
Punta Abreojos, B.C.S., Mexico	24°41′, 113°34′	Jun 1999		2	1	
San Juanico, B.C.S., Mexico	24°14′, 112°27′	Oct 1999		2	1	
Bahía Magdalena, B.C.S., Mexico	24°46′, 112°06′	Dec 1999	2	2	1	
Panulirus inflatus (blue spiny lobster)						
Bahía Magdalena, B.C.S., Mexico	24°46′, 112°06′	Nov 2001	3	3	1	21
Mazatlán, Sin., Mexico	23°13′, 106°26′	Aug 2002	4	3	1	14
Las Peñitas-Sayulita, Nay., Mexico	21°00′, 105°23′	Aug 2002	2	3	1	24
Barra de Navidad, Jal., Mexico	19°12′, 104°42′	Jan 2005	1	3	1	15
Zihuatanejo, Gue., Mexico	17°37′, 101°33′	May 2005		2	1	29
Puerto Angel, Oax., Mexico	15°39′, 96°29′	Nov 2002			1	23
Panulirus gracilis (green spiny lobster)						
Bahía Magdalena, B.C.S., Mexico	24°46′, 112°06′	Nov 2001	2			
Mazatlán, Sin., Mexico	23°13′, 106°26′	Aug 2002	3	4	2	21
Las Peñitas-Sayulita, Nay., Mexico	21°00′, 105°23′	Aug 2002	1	5	1	23
Punta Maldonado, Gue., Mexico	16°20′, 98°34′	May 2005		5	2	25
Total			20	42	18	195

PCR was performed in a total volume of 25 μL (Invitrogene 1× PCR buffer, 0.2 mM dNTP mix, 0.48 μM of each primer, 4.0 mM $MgCl_2$, 1.25 U *Taq* DNA polymerase), with an iCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA). The PCR program consisted of a denaturation step at 94°C for 2 min, followed by 30 cycles of 1 min at 94°C, 1 min at 60°C, and 2 min at 72°C, followed by a final extension step of 4 min at 72°C. PCR products were confirmed using electrophoresis on 1% agarose gel, along with a molecular weight marker to estimate the fragment size. The gel was stained with SybrGold (Molecular Probes, Eugene, OR). All amplified products were sequenced with primers 16Sar-L and 16Sbr-H (Macrogen, Korea) and deposited in GenBank (accession numbers EF546597 through EF546616).

The 16S rRNA gene sequences were aligned and edited with the program Sequencher, vers. 4.5. (Gene Codes Corporation, Ann Harbor, MI). A neighbor-joining phylogram of haplotypes, based on the Kimura-2 parameter model, was constructed in Molecular Evolutionary Genetics Analysis (MEGA) software vers. 3.0 (Kumar et al., 2004). Three sequences (one for each species) were used for the identification of diagnostic restriction enzyme recognition sites in ChromasPro software, vers. 1.33 (Technelysium Pty Ltd, Tewantin, Queensland, Australia). Even though several restriction enzymes revealed various cut sites in the sequences, we selected only those that were easily seen on agarose gel. To check for consistency of the restriction enzyme cutting pattern in each species, the analysis was done with 17 additional sequences.

The RFLP pattern of the selected enzymes of another 42 adult lobster specimens of the three species was tested for specimens collected at different locations (Table 1). Each digestion reaction occurred in a final 7μL volume with 0.7 unit/μL of the selected enzyme, 3.5 μL PCR product, and according to the manufacturer's instructions (New England Biolabs, Ipswich, MA). Reaction products for each enzyme were incubated for eight hours at the optimal temperature suggested by the manufacturer. For electrophoresis, restricted products were run on 2% agarose gels and stained with SybrGold. A molecular weight marker was added to the agarose gel to estimate fragment size. To check consistency of intra- and interspecific nucleotide variation in addition to the 20 sequences, 195 sequences (a total

of 215) from different geographical sites of *P. inflatus* (n=126) and *P. gracilis* (n=69) were analyzed. This extension was important because the larvae of these species are found in the same area and there is a higher possibility that misidentification will occur when using only morphological criteria (Table 1).

Panulirus penicillatus (pronghorn spiny lobster) is found along the Pacific coast of Mexico and even though it is found in small numbers, it could be confused with the larvae of the other species. The restriction pattern of a 16S rRNA gene sequence reported in the GenBank (accession number AF337974) was compared to those of the other three lobster species.

Multiplex PCR analysis

Species-specific primers for *P. interruptus* have been described in a previous study (García-Rodriguez and Perez-Enriquez, 2006; GenBank accession number EF565146). Based on this sequence, a reverse primer (5′-TGGTGTGATCCCGTTACTTG) was designed to amplify a ~1250 base-pair (bp) mtDNA fragment containing the 12S rRNA gene and the control region in *P. inflatus* and *P. gracilis* by means of Weider et al.'s (1996) forward primer (srRNA: 5′-CAGGGTATCTAATCCTGGTT). PCR thermal cycling consisted of an initial denaturation of 2 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 58°C (for *P. inflatus*, but 52°C for *P. gracilis*), 2 min at 72°C, and a final cycle of 4 min at 72°C. PCR products from adult specimens of each species were sequenced by using the srRNA primer. The Primer3 program (Rozen and Skaletsky, 2000) was used to design specific primers for each species; these primers would have similar melting temperatures but products of different sizes (Fig. 1). Sequences obtained with specific primers were deposited in GenBank (accession numbers EF565144 and EF565145).

The specificity and reliability of the multiplex PCR reaction with 18 previously identified adult lobsters collected in different regions were tested. For *P. interruptus* (n=7), specimens were obtained from California, Isla Guadalupe, Baja California, and Baja California Sur; for *P. inflatus* (n=6), specimens were collected from Baja California Sur, Sinaloa, Nayarit, Jalisco, Guerrero, and Oaxaca; for *P. gracilis*, specimens (n=5) came from Sinaloa, Nayarit, and Guerrero (Table 1).

Multiplex PCR reactions were carried out in a total volume of 12.5 μL, mixing 0.48 μM of each primer (one common primer: srRNA, and the three species-specific primers: LanCR-R (5′-AAAAATTCAGGCTAATGGA), PinRC1-b (5′-GATGGCCCATTACCGAACTA), and PgraRC1-b (5′-TTGTGAAACGTCTGTTTACATTTATTT)), Invitrogene 1× PCR buffer, 0.2 mM dNTP mix, 4.0 mM $MgCl_2$, and 0.625 U Taq DNA polymerase. PCR thermal cycling consisted of an initial denaturation of 2 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 59°C, 2 min at 72°C, and a final cycle of 4 min at 72°C. The amplified products from each species were distinguished by electrophoresis on 1.0 % agarose gels.

Application of molecular markers

Phyllosoma larvae were collected during an oceanographic cruise outside the Gulf of California in November 2004. Plankton sampling consisted of horizontal surface tows of a neuston collection net at 3.5 knots (6.4 km/h) for 5 min (see González-Armas et al., 1999). Sampling gear consisted of a rectangular plankton net of Nytex with 505-μm mesh; it had a 30×50 cm mouth area and the net was 3 m long. Phyllosoma larvae were sorted by hand after each tow and fixed with 70% ethanol.

A fragment of the pereiopods, antenna, or eyes was obtained from each lobster larva for DNA isolation by lysing the tissue in 15 μL lysis buffer (10 mM Tris-HCL, pH 8.3, 50 mM KCl, 0.5% Tween-20), and 1.87 μL (4 μg/μL) proteinase *K* and incubated overnight at 55°C. Each reaction was then maintained at 95°C for approximately 10 min and stored at 4°C until analysis. Steps of PCR-RFLP and PCR multiplex for larval lobsters were the same as those carried out for adult specimens.

Before genetic analysis, 46 lobster larvae collected from the Pacific Ocean were categorized into four possible groups based on morphological criteria (Johnson and Knight, 1966; Johnson, 1971; Báez, 1983): group 1) *Panulirus inflatus*-like (n=17); group 2) *Panulirus gracilis*-like (n=8); group 3) *Panulirus inflatus-gracilis*-like (n=12); and group 4) *Panulirus*-like (n=9).

Results

PCR-RFLPs

The 16S rRNA gene fragment was correctly amplified in the three lobster species. The size of the 16S rRNA gene fragment amplified in the three spiny lobster species was estimated at 563 bp and did not differ in size among species. There was wide inter- and intraspecific variation in nucleotide sequences among the lobster species, indicating the potential for species discrimination. A haplotype tree showed the aggregation of haplotypes according to each species (Fig. 2).

From the analysis of 20 sequences, two restriction enzymes (*Bsm*AI, GTCTCN′ and *Hin*fI, G′ANTC) were selected that allowed discrimination among the three species (Fig. 3). The restriction products of the *Bsm*AI digests were two fragments (~401 and ~162 bp) in both *P. interruptus* and *P. inflatus* (named "haplotype A") and three fragments (~401, ~115, and ~47 bp) in *P. gracilis* (named "haplotype B"). Restriction digests that used *Hin*fI produced two fragments (~440 and ~ 123 bp) for *P. interruptus* and most *P. gracilis* specimens (named "haplotype A"), but did not cut *P. inflatus* (named "haplotype B"). One *P. gracilis* sequence appeared to be haplotype B due to the absence of the *Hin*fI site. The composite haplotypes were constructed by the combination of the haplotype names of each enzyme, resulting in AA for *P. interruptus*, AB for *P. inflatus*, and either BA (98.7%) or BB (1.3%) for *P. grac-*

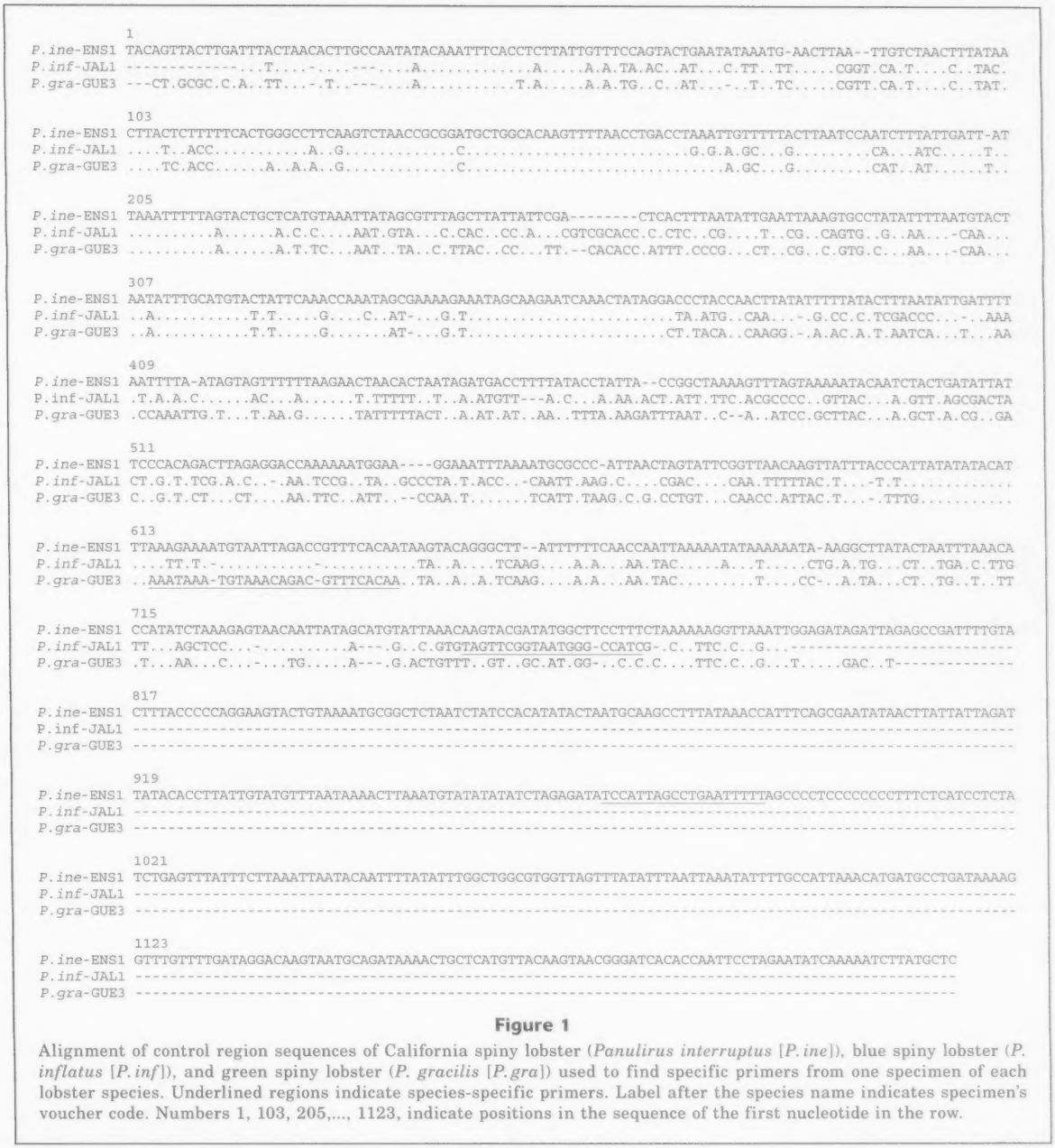

Figure 1

Alignment of control region sequences of California spiny lobster (*Panulirus interruptus* [*P. ine*]), blue spiny lobster (*P. inflatus* [*P. inf*]), and green spiny lobster (*P. gracilis* [*P. gra*]) used to find specific primers from one specimen of each lobster species. Underlined regions indicate species-specific primers. Label after the species name indicates specimen's voucher code. Numbers 1, 103, 205,..., 1123, indicate positions in the sequence of the first nucleotide in the row.

ilis (Table 2). Thus the composite haplotypes are able to delineate among these species. All electrophoretic patterns of PCR-RFLP products of adult specimens were congruent with those obtained from sequences analysis. Finally, the sequence of *P. penicillatus* had restriction sites that would produce different patterns from the other species (Fig. 3).

Multiplex PCR

A high annealing temperature (59°C) was adequate for successful multiplex PCR reactions containing the DNA of the three species. There were no additional fragments to those expected that could prevent identification of lobster species in all tested adult specimens. The size of

Table 2

Mitochondrial DNA (mtDNA) 12S rRNA-control region (563 base pairs [bp]) fragment sizes resulting from the *Bsm*AI and *Hin*fI-recognizing sites during nucleotide sequence analyses of three *Panulirus* species: California spiny lobster (*Panulirus interruptus*), blue spiny lobster (*P. inflatus*), and green spiny lobster (*P. gracilis*). Hap = haplotype nomenclature (see text for explanation); % = percentage of haplotypes in a sample of 215 sequences. Composite haplotypes were obtained by combining the haplotype of each enzyme.

Species	Enzyme	Fragment size (bp)	Hap	%	Composite haplotype
P. interruptus	*Bsm*AI	162,401	A	100.0	AA
	*Hind*I	440,123	A	100.0	
P. inflatus	*Bsm*AI	162, 401	A	100.0	AB
	*Hind*I	563	B	100.0	
P. gracilis	*Bsm*AI	115,47,401	B	100.0	BA / BB
	*Hinf*I	440,123	A	98.7	
		563	B	1.3	

the amplified product delineated all three species in a 1% agarose gel. The fragment size of *P. interruptus* (with the use of LanCR-R primer) was ~1000 bp, the fragment size of *P. inflatus* (with the use of PinRC1-b) was ~800 bp, and the fragment size of *P. gracilis* (with PgraRC1-b) was ~700 bp (Fig. 4). Even though two *P. interruptus* specimens produced unspecific amplifications, the fragments stained weakly compared to the 1000-bp fragment and did not interfere with identification. Because the specimens analyzed by multiplex PCR were collected at different sites, these data show that there is no apparent intraspecific variation, which indicates that the multiplex primer set provides a method that can be used to identify the three lobster species.

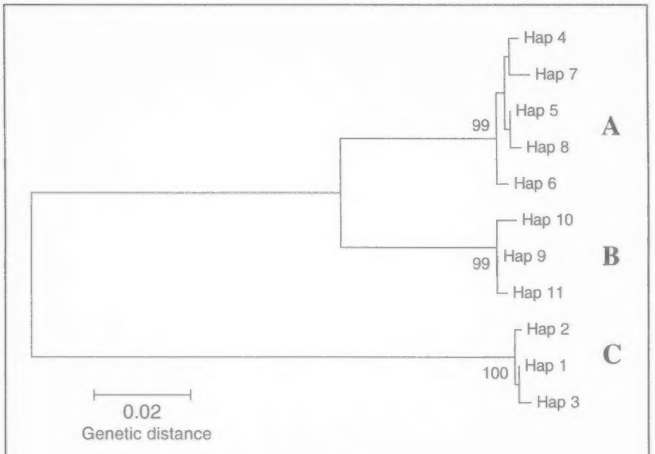

Figure 2

Neighbor-joining phylogenetic tree based on the mitochondrial 16S rRNA gene haplotypes (Hap 1–11) from a total of 20 adult lobsters of blue spiny lobster (*Panulirus inflatus*), green spiny lobster (*P. gracilis*), and California spiny lobster (*P. interruptus*). Haplotype groups identified as A, B, and C represent blue, green, and California spiny lobster, respectively. Tree reconstruction was based on Kimura's two-parameter distance (K2P) with 1000 replications. Numbers on the nodes are bootstrap values. The branch length is measured as the number of nucleotide substitutions.

Larval identification

Species identification based on PCR-RFLP did not support the identification based on morphological criteria. The fragment patterns produced by digestion with *Bsm*AI and *Hin*fI provided a means for identifying *P. inflatus* and *P. gracilis*. Two specimens in each of groups 1 and 2 were identified as *P. gracilis*. The 42 remaining larvae belonging to groups 1, 2, 3, and 4 were identified as *P. inflatus*; none of the specimens were identified as *P. interruptus*. Multiplex PCR analysis confirmed the results.

Discussion

Several methods with molecular markers have been carried out for identification at the species level. Even though PCR-RFLP and multiplex-PCR are widely used as tools in distinguishing species of different taxonomic groups (Moore et al., 2003; Chow et al., 2006a), efforts have to be made to obtain the proper amplification primers when universal primers do not give consistent results in the studied species.

According to our analysis, identification of spiny lobster species can be successfully done by combining different strategies. First, although insufficient for recognizing spiny lobsters larvae, morphological criteria should be used. Then, one or both of the genetic techniques can be applied to definitively support the morphological results. The simultaneous use of the PCR-RFLP and multiplex PCR

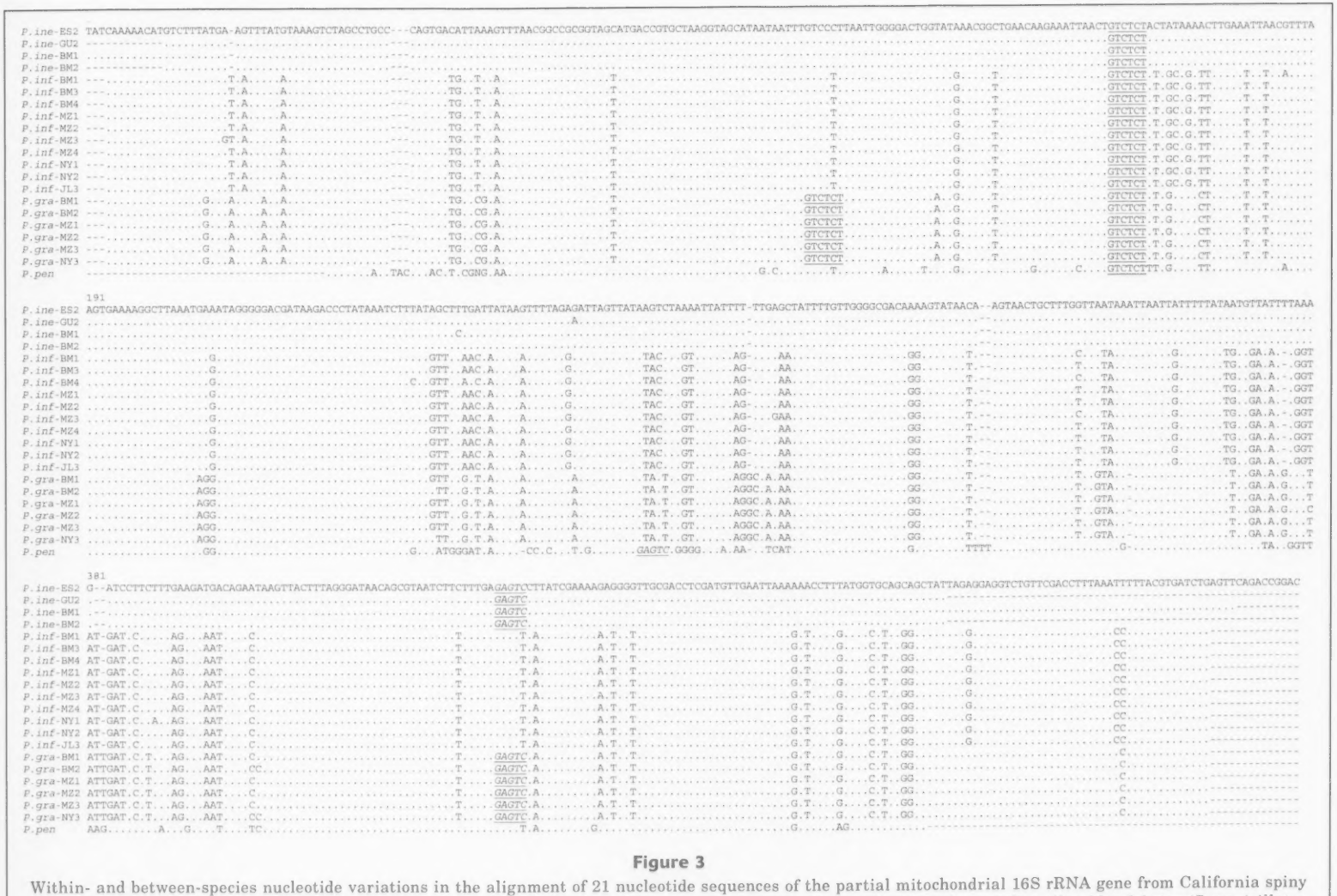

Figure 3

Within- and between-species nucleotide variations in the alignment of 21 nucleotide sequences of the partial mitochondrial 16S rRNA gene from California spiny lobster (*Panulirus interruptus* [*P.ine*]), blue spiny lobster (*P. inflatus* [*P.inf*]), green spiny lobster (*P. gracilis* [*P.gra*]), and Hawaiian blue lobster (*P. penicillatus* [*P.pen*]). *Bsm*AI-recognition sites are underlined. *Hin*fI-recognition sites are underlined and italicized. Dots indicate the same nucleotide as that in the sequence of specimen *P. ine*-ES2; hyphens indicate sequence gaps. The label after the species name indicates specimen's voucher code. Numbers 1, 191, and 381 indicate positions in the sequence of the first nucleotide in the row.

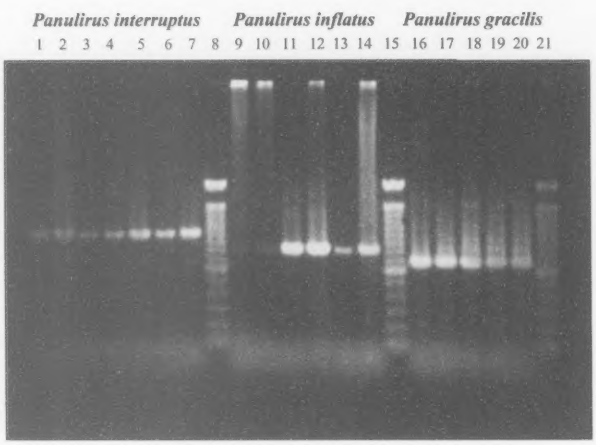

Figure 4

Polymerase chain reaction (PCR) products with the multiplex-PCR method for each of the three lobster species: California spiny lobster (*Panulirus interruptus*), blue spiny lobster (*P. inflatus*), and green spiny lobster (*P. gracilis*). Lanes 8, 15, and 21 are 100-bp ladder molecular markers.

techniques is recommended because there are instances for which unsuccessful amplifications are obtained by one of the methods. This is a consequence of mutations in the annealing sites, rather than a failure of PCR techniques (Ray et al., 2002).

The 20 sequences that we evaluated for differences in restriction patterns revealed species-specific restriction sites for *Bsm*AI and *Hin*fI. Amplified products of previously identified adult lobsters were concordant with the restriction patterns found in these sequences. The PCR-RFLP technique is successful for separating *Panulirus* larvae because 16S rRNA is relatively easy to amplify at intraspecific conservative sites and interspecific variable sites.

The PCR-RFLP has also been applied to the cytochrome oxidase I (COI) gene to identify 10 spiny lobster species of the genus *Panulirus* found in the northwestern Pacific Ocean (Chow et al., 2006a). In general, Chow et al. (2006a) found more than two haplotypes per species; however, no composite haplotype was shared by these species. Although Chow et al. (2006a) suggested increasing efforts in searching for intraspecific variation on larger samples, the probability of misidentification is very low because substantial divergence has been observed among species of the genus *Panulirus* (Ptacek et al., 2001). Our results support the finding of high divergence among the three lobster species in Mexico on the basis of nucleotide intraspecific variation (Fig. 2).

Multiplex-PCR fragments are clearly capable of discriminating among the three lobster species. This method is fast, simple, and relatively inexpensive because species identification can be performed by using just PCR amplifications with no digestion and with small reaction volumes. The multiplex-PCR method used in this study is successful because it is based on species-specific primers and a sufficiently high annealing temperature (Tm) of 59°C is used that avoids the amplification of unspecific PCR products. Also, a high Tm reduces the possibility of amplifying homologous sequences from other species.

Identification of lobster larvae is consistent with the restriction patterns found in adult lobsters and identifications carried out by PCR-RFLP can be correctly confirmed by using multiplex PCR. Molecular analyses showed that the previous larval classification (based on morphological characters) was probably incorrect because very small or injured specimens were used. Alternatively, misidentification in lobster larvae could also be a consequence of morphological criteria that are not diagnostic characters for discriminating between lobster larvae, as it has been reported in other *Panulirus* species, such as the Japanese spiny lobster (*P. japonicus*) and species from the Atlantic Ocean (i.e., the Caribbean spiny lobster, *P. argus*) (Silberman and Walsh, 1992; Chow et al., 2006a, 2006b). Morphological criteria currently used for the identification of phyllosoma larvae of *P. interruptus*, *P. inflatus*, and *P. gracilis* are insufficient as well. Further descriptions of larvae specimens should be conducted to search for consistent morphological differences between species.

Other possible applications of PCR-RFLPs and multiplex PCR are used in fishery forensics, when lobster

products must be identified. This is especially the case when lobster products of unknown origin have to be analyzed if there is suspicion that the lobster was obtained by illegal fishing, as in the case of important protected species, such as sea turtles (Moore et al., 2003).

Conclusions

We used the nucleotide variation of two mtDNA fragments from adult spiny lobster samples to find molecular marker applications for species discrimination. The RFLP and multiplex-PCR protocols developed in this study allow for correct discrimination of three commercial lobster species inhabiting the Pacific coast of Mexico. Application of both types of molecular markers in the identification of lobster larvae showed concordance and the potential to discriminate between phyllosoma species during early stages. Molecular identification of larvae was inaccurate with our previous assignment based on morphological criteria. Thus, the use of morphological characteristics in phyllosoma larvae can be misleading for identification to the species level because anatomical parts can easily be damaged during collection. This new information on genetic identification of species at the larval stage is of wide interest because studies focused on taxonomic and ecological revisions require accurate species identification.

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Advantages of using crest nets to sample presettlement larvae of reef fishes in the Caribbean Sea

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Identifying the spatial and temporal patterns of larval fish supply and settlement is a key step in understanding the connectivity of meta-populations (Sale et al., 2005). Because of the potentially dispersive nature of the pelagic larval phase of most reef fishes, tracking cohorts from hatching to settlement is extremely difficult (but see Jones et al., 1999). However, for many studies it is sufficient to sample larvae immediately before settlement. Many coral reef fish species use mangrove and seagrass beds as nursery habitats (Nagelkerken et al., 2001; Mumby et al., 2004) and larvae of these species must pass over the reef crest in order to arrive at their preferred settlement habitats. The ability to sample this new cohort of larval fishes provides opportunities for researchers to explore the intricacies of the transition from larva to juvenile (Searcy and Sponaugle, 2001). Quantifying the potential settlers also provides valuable information about the spatial and temporal supply of presettlement larvae (Victor, 1986). Therefore a number of larval sampling methods were developed, one of which is the use of crest nets (Dufour and Galzin, 1993).

Crest nets are rigid-frame tapering nets that are fixed to the substrate in shallow water immediately behind the crest of the reef (see Doherty and McIlwain, 1996 for full description). The top of the crest net is above the surface of the water and currents and wave action force larvae into the mouth of the net. Because of the turbulence of the water coming over the reef crest and the fact that the whole water column is filtered, net avoidance by larval fishes is estimated to be minimal. Channel nets (Shenker et al., 1993) and light traps (Doherty, 1987), on the other hand, remain the dominant methods for sampling settlement-stage larval fishes on western Atlantic reefs. Surface channel nets are floating nets that are free to swivel with the prevailing current. Where crest nets are positioned in the shallow back reef, channel nets are positioned in deeper channels between mangroves, further away from the reef. Crest nets have been widely used in the Pacific Ocean to quantify the larval abundance of coral reef fishes immediately before settlement (Leis et al., 1998; Dufour et al., 2002; Leis et al., 2003; McIlwain, 2003; Lecchini et al., 2004). Despite the apparent success of sampling reef fishes in the Pacific Ocean with crest nets, there are currently no reports of crest nets being employed for sampling reef fishes in the Caribbean Sea. The first objective of this study was to simultaneously deploy crest and channel nets to compare the abundance and species richness of larval fishes sampled. It was hypothesized that crest nets would capture more larvae by sampling the whole water column on the reef crest as opposed to channel nets that sample only surface waters.

Larval reef fish possess impressive swimming capabilities (Leis and Carson-Ewart, 1997) and have the ability to detect reefs at a distance (Myrberg and Fuiman, 2002) and can therefore influence their own dispersal. However, many other abiotic factors can still influence their growth, survival, transport, and eventual arrival at a suitable settlement habitat. The abundance of larvae present is related to lunar period in some areas (Robertson et al., 1988, Thorrold et al., 1994; Sponaugle and Cowen, 1996), but this abundance is not fully correlated with peaks in abundance in other areas (Kingsford and Finn, 1997). Larval growth rates and swimming ability vary with water temperature in some species (Green and Fisher, 2004) and winds can alter the strength and direction of supplying currents. The second objective of this study was to explore correlations between certain abiotic factors (lunar phase, water temperature, and prevailing wind) and the number of species and individuals collected by each net type.

Materials and methods

Study site

Fieldwork was conducted at Turneffe Atoll, Belize (17°16′5″N, 87°48′57″W, Fig. 1A). Turneffe Atoll is part of the Meso-American Barrier Reef System (MBRS) that runs along southern Mexico through the waters of Belize, Guatemala, and Honduras. The MBRS is the world's second largest coral reef system after the Great Bar-

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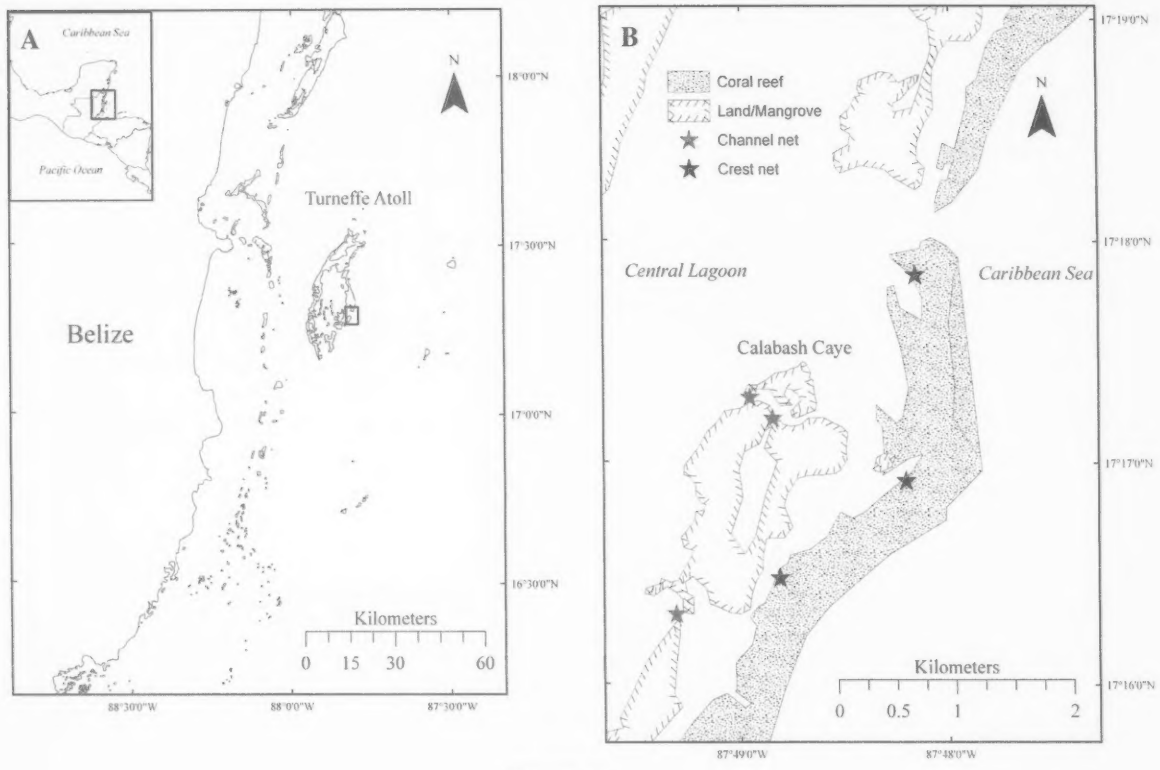

Figure 1

Maps indicating the position of Turneffe Atoll, Belize (**A**) and the positions of crest and channel nets around Calabash Caye (**B**). The inset in map A indicates the position of Belize in Central America and the rectangle around Turneffe Atoll specifies the area enlarged in map B.

rier Reef in Australia. Turneffe Atoll is a large offshore ring of islands bordered by coral reefs. It has a large central lagoon that contains many mangrove islands and channels. The atoll is located outside the coastal barrier reef, approximately 46 km west of mainland Belize (Fig. 1A).

Larval collection and identification

The definnition of "larva" will follow that of Leis (2006): the posthatching pelagic life history stage of demersal fishes (which is equivalent to the presettlement stage of Kingsford and Milicich, 1987). Larvae were sampled with crest nets and channel nets from 6 July to 26 August 2005, 24 January to 4 March 2006, and 17 May to 28 July 2006. One crest net was positioned in shallow water directly behind the reef crest in each of three sites approximately 1 km apart (Fig. 1B). The crest nets had a mean width of 5.85 m, a mesh size of 2 mm, and were situated in 65–90 cm of water at each site. One surface channel net (Shenker et al., 1993) was placed in each of three separate mangrove channels leading to the central lagoon, each net with a square mouth (2 m × 1 m) with 1.6-mm mesh. It was not our intent to optimize the performance of either net. Therefore, although there were differences in net cross-sectional area, mesh sizes, and placement locations between crest and channel nets, these differences represent how each net has been typically deployed.

In preliminary sampling at Turneffe Atoll, near zero or zero catches occurred during daylight hours, which was consistent with the findings of Shenker et al. (1993). Therefore, collections were made only at night. Both types of nets were deployed nightly and the catch was retrieved and identified each morning. All individuals of all species of larval reef fishes were counted each day. Where species could not be determined, the lowest taxonomic category that could be unambiguously determined was used. Larvae were examined live and identified (Humann and DeLoach, 2002; Richards, 2005). Over the course of the study a number of specimens of all species were preserved in 95% ethanol for later validation.

Environmental variables

Mechanical flowmeters (model 2030R6, General Oceanics, Inc., Miami, FL) were deployed with each net. These

meters are equipped with a high-resolution rotor for low-speed flow and had a minimum threshold of approximately 6 cm/sec. The mean nightly measurement of flow was used to calculate the total volume of water filtered by each net. Underwater temperature loggers (Hobo Pendant Temperature Logger, Onset Computer Corp., Bourne, MA) provided a fine-scale record of the temperature of water being sampled (temperature data were not available for 2005). Wind speed and wind direction data were obtained from an automated weather station at Belize City International airport (17°53′N, 88°30′W). These wind reports provided a reasonable record of prevailing conditions at Turneffe Atoll because of the proximity and lack of geographic obstacles between the two points. The mean nightly wind direction was given a positive value for an on-shore wind and a negative value for an off-shore wind. Finally, a variable incorporating both the nocturnal illumination and tidal periodicity of the lunar cycle was calculated (see D'Alessandro et al., 2007). The hours of nocturnal flood tides were calculated for each sampling night with tide prediction software (JTide, vers. 5.1, P. Lutus, freeware software available online) and this number was multiplied by the percentage of the moon that was visible (full moon=100%).

Statistical analyses

Species-environment ordinations (CANOCO, vers. 4.5, Microcomputer Power, Ithaca, NY) were used to establish the relative importance of individual environmental factors (sampling season, wind, water temperature, and nocturnal flood tides) in explaining the overall variance in larval abundance and species richness in the catch. The species and environmental data were found to be linear and were examined by redundancy analysis (RDA). An RDA plot shows the best fit of multivariate data in a two-dimensional ordination.

The temporal supply of fish larvae was investigated by using correlation plots and circular statistics (Rayleigh z; Zar, 1984). Cross-correlation plots were used to compare the timing of the capture of larvae in the two different environments, namely behind the reef crest where crest nets were used and the mangrove channels where channel nets were used. Once both net types were shown to collect larvae synchronously (see *Results*), the data for both nets were combined into a single time series. Auto-correlations were then plotted to examine the temporal periodicity of the catch. To achieve this, all three sampling periods were concatenated into a continuous time series to ensure that more than 2.5 continuous lunar cycles were included (the minimum necessary for auto-correlation analysis for an examination of lunar periodicity). Each day was assigned a number corresponding to its point in the lunar cycle (lunar days 1–29, 1=new moon). To ensure that the cycles were continuous, any overlapping lunar days between the sampling periods were deleted (from the middle period, spring 2006). The final time-series had 164 days, from which 14 overlapping days were deleted.

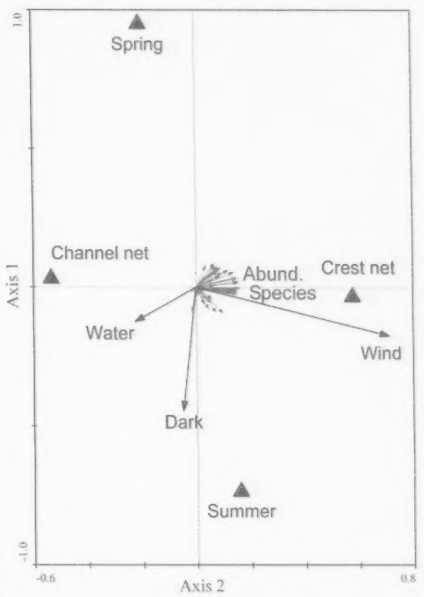

Figure 2

Redundancy analysis plot of flow-corrected data. The angle between two variables represents the correlation between them (0°=positive correlation, 90°=no correlation, 180°=negative correlation), and the length of the arrow represents the magnitude, i.e., the longer the arrow, the greater the correlation coefficient. Environmental variables are shown as labeled arrows. Water = water temperature, Dark = hours of moonless, nocturnal flood tide, and Wind = average speed (km/h) of onshore wind. Categorical variables are shown as triangles. Species are shown as unlabelled grey arrows; individual species names have been omitted. Abundance and species richness are shown as black arrows labeled as Abund. and Species, respectively.

Results

A total of 53,579 larval reef fishes were caught that represented 33 families and 59 identified species (Table 1). On an average night, a crest net trapped 166.3 larvae (standard deviation [SD]=407.4) and 8.5 species (SD=5.8), whereas a channel net trapped 4.1 larvae (SD=12.2) and 0.9 species (SD=1.5). See Table 1 for list of families and species sampled by both net types.

Ordinations

There was a strong distinction between the species assemblages caught in the two net types (Fig. 2). Only data for 2006 sampling periods are presented in Figure 2, as no water temperatures were available for 2005

Table 1

Total number of fish larvae sampled with crest and channel nets at Turneffe Atoll, Belize, during the three sampling periods (summer 2005, spring 2006, and summer 2006). Barred lutjanids refers to *Lutjanus apodus*, *L. analis*, *L. cyanopterus*, *L. griseus*, and *L. jocu*. Striped *Stegastes* refers to *Stegastes diencaeus*, *S. leucostictus*, and *S. variabilis*.

			Summer		Spring		Summer		
			2005		2006		2006		
Family	Genus	Species	Crest net	Channel net	Crest net	Channel net	Crest net	Channel net	Total
Acanthuridae	*Acanthurus*	*bahianus*	1	0	0	0	8	0	9
	Acanthurus	*chirurgus*	5	0	0	0	1	0	6
	Acanthurus	*coeruleus*	10	0	2	0	8	0	20
Achiridae	*Achirus*	sp.	0	0	0	0	3	0	3
Antennariidae	*Histrio*	*histrio*	5	1	0	0	2	0	8
		All others	7	0	1	0	8	0	16
Apogonidae	*Apogon*	*maculatus*	302	26	53	6	464	10	861
	Apogon	*quadrisquamatus*	207	1	15	0	63	0	286
	Astrapogon	*puncticulatus*	114	5	261	0	212	1	593
Aulostomidae	*Aulostomus*	*maculatus*	0	0	2	0	1	0	3
Labrisomidae	*Starksia*	spp.	534	0	20	0	270	0	824
Bothidae	*Bothus*	spp.	43	18	8	4	28	5	106
Callionymidae	*Paradiplogrammus*	*bairdi*	123	0	25	0	924	1	1073
Carangidae		All species	20	2	9	1	13	0	45
Chaetodontidae	*Chaetodon*	*capistratus*	25	1	0	0	7	0	33
	Chaetodon	*ocellatus*	5	0	0	0	0	0	5
Cynoglossidae	*Symphurus*	spp.	129	0	29	0	6	0	164
Diodontidae	*Chilomycterus*	*antennatus*	5	0	0	0	0	0	5
Elopomorpha		All species	471	221	627	251	1759	173	3502
Gerreidae	*Eucinostomus*	spp.	13,450	296	1557	21	10,592	17	25,933
Gobiesocidae	*Arcos*	*rubiginosus*	0	0	0	0	3	0	3
Gobiidae	*Bathygobius*	*curacao*	1	0	0	0	177	0	178
	Ctenogobius	*saepepallens*	0	0	97	1	13	0	111
	Gnatholepis	*thompsoni*	2043	0	882	0	2623	12	5560
	Priolepis	spp.	23	0	5	0	226	0	254
	Unknown	spp.	3503	1	329	0	1108	0	4941
Labridae	*Halichoeres*	spp.	296	0	23	2	184	0	505
	Thalassoma	*bifasciatum*	21	0	3	0	31	0	55
	Xyrichtys	spp.	83	0	232	1	30	1	347
Lutjanidae	Barred lutjanids	All	150	12	8	1	18	2	191
	Lutjanus	*synagris*	0	1	4	0	1	0	6
	Lutjanus	*mahogoni*	4	0	0	0	0	0	4
	Ocyurus	*chrysurus*	2	0	6	0	0	0	8
Microdesmidae		All species	39	1	52	7	63	4	166
Monacanthidae	*Cantherines*	sp.	1	0	4	0	1	0	6
	Monacanthus	*ciliatus*	184	2	0	0	23	4	213
	Monacanthus	*tuckeri*	113	8	22	1	105	9	258
Ogcocephalidae	*Ogcocephalus*	*nasutus*	3	0	0	0	6	0	9
	Halieutichthys	*aculeatus*	4	0	0	0	2	0	6
Ophidiidae		All species	5	0	5	0	21	0	31
Ostraciidae	*Lactophrys*	spp.	77	1	32	0	3	0	113
Paralichthyidae	*Syacium*	spp.	0	0	11	0	4	8	23
Pomacanthidae	*Pomacanthus*	spp.	2	0	3	0	2	0	7
Pomacentridae	*Abudefduf*	*saxatilis*	13	0	6	0	3	2	24
	Microspathadon	*chrysurus*	0	0	0	0	1	0	1

continued

Table 1 (continued)

Family	Genus	Species	Summer 2005 Crest net	Summer 2005 Channel net	Spring 2006 Crest net	Spring 2006 Channel net	Summer 2006 Crest net	Summer 2006 Channel net	Total
Pomacentridae (continued)	*Stegastes*	*adustus*	3	0	0	0	1	0	4
	Stegastes	*partitus*	2	1	0	0	23	1	27
	Striped *Stegastes*	All	171	2	0	1	258	12	439
Scaridae	*Sparisoma*	spp.	329	3	359	3	838	0	1532
Scorpaenidae	*Scorpaena*	spp.	44	2	202	1	64	2	315
Serranidae	*Diplectrum*	spp.	45	5	0	0	5	0	55
	Pseudogramma	*gregoryi*	95	0	38	0	117	0	250
	Rypticus	sp.	2	0	0	0	5	0	7
	Hypoplectrus	spp.	0	0	0	0	7	0	7
Sphyraenidae	*Sphyraena*	*barracuda*	125	13	6	0	39	6	189
Syngnathidae	*Cosmocampus*	spp.	341	1	111	0	95	0	548
Tetraodontidae	*Sphoeroides*	spp.	0	0	7	3	16	0	26
	Canthigaster	spp.	435	0	24	7	26	0	492

(when 2005 data were analyzed separately, a very similar plot was obtained). Most species were captured in greater abundance with crest nets and rarely, if ever, caught in the channel nets. For example, the families Acanthuridae, Ogcocephalidae, and Pomacanthidae were only caught in crest nets and there were no species or families that were exclusively caught in channel nets. The summer and spring sampling periods were extremely different (Fig. 2). However, when the three sampling periods were plotted separately, very similar ordinations with respect to environmental factors were obtained. The difference between summer and spring in the combined ordination of Figure 2 could be due to the lower numbers of larvae captured in spring 2006; however, there were notable absences of families in that sampling period, e.g., no Chaetodontidae or Ogcocephalidae and only a single representative of Pomacentridae.

Of the environmental variables (Fig. 2), the onshore wind was positively correlated with abundance and species richness of larval reef fishes sampled in crest nets. The combined factor (nocturnal illumination and tidal periodicity) was important but did not align strongly with the other explanatory or species variables. Higher water temperatures at the net sites corresponded with fewer larvae caught because water temperature was negatively correlated with the presence of the vast majority of species.

Time series analyses

Peaks and lows in the supply of fish larvae appeared on the same nights in reef crest nets and channel nets in the mangroves (Fig. 3). The cross-correlation plots between net types revealed that catches (both in terms of abundance and species richness) were significantly correlated at a lag of zero (data sets were aligned for correlation on the same day at a lag of zero, one data set leads the other by one day for correlation at a lag of +1, etc.). For abundance, the greatest correlation between net types was at a lag of zero days (Fig. 3A). A lesser correlation at a lag of plus three days indicates that some groups of larvae took three days to pass from the reef crest to the mangrove channels. The other significant correlations at lags of −4, −3, and −1 days are more difficult to explain. There seems to be no biological reason that cohorts of reef fish larvae should arrive in the mangrove channels up to four days before they arrive at the reef crest. This finding may be a result of pooling abundances of all species and could possibly be resolved with further analysis by splitting abundances into families or species (where possible). Species richness was also correlated at a lag of zero days; however, the other significant correlation, at a lag of −4 days, was greater than that at day zero (Fig. 3B). As with abundance, there seems to be no biological explanation for this correlation and more detailed analysis may prove advantageous.

The auto-correlation plot for abundance (Fig. 4A) illustrates that there was no periodicity in the flow-corrected data and that the catch on any one night was not correlated with that on the preceding or following nights. However, the plot for species richness (Fig. 4B) shows a lunar periodicity in the numbers of species caught. The significant negative correlation at a lag of 16 days (at just over half the lunar cycle) shows that greater numbers of species caught in new-moon periods

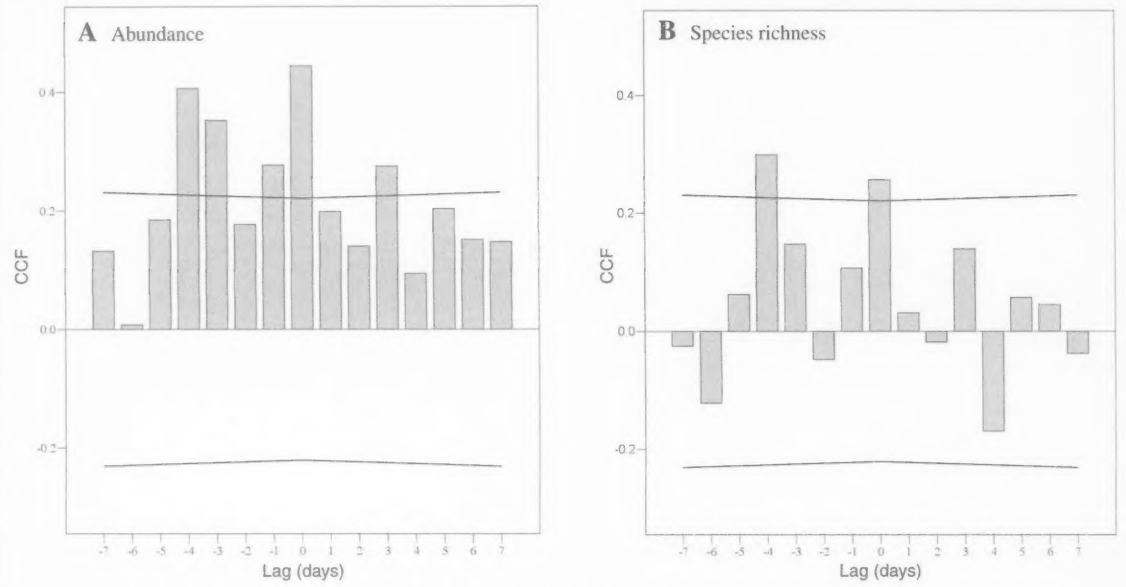

Figure 3

Cross-correlation plots of the average nightly abundance (**A**) and species richness (**B**) for larvae sampled in crest and channel nets for flow-correccted data. These plots identify any significant delay between catch in the crest versus channel nets. Lag refers to the number of days by which one of the data sets is offset from the other when the correlation is calculated; data sets are aligned for correlation on the same day at a lag of zero, and the crest net data leads the channel net data by one day for correlation at a lag of +1, etc. The cross-correlation function (CCF, correlation coefficient between the two data sets at each lag) is on the ordinate. Values of the CCF above and below zero represent positive and negative correlations between net types; the horizontal lines above and below the abscissa indicate the upper and lower 95% confidence limits, respectively.

corresponded to fewer numbers of species caught in full-moon periods.

Discussion

Crest nets caught greater numbers of individuals and species per deployment than channel nets and would therefore be an advantageous sampling tool to use in studies attempting to maximize the chance of catching greater numbers of a certain species. However, the difference between net types was not solely due to the design of the net. The two net types were deployed at two different habitats. All larvae passing over the top of a small width of the reef crest were sampled as the reef slope forced them into a constrained water column. In contrast, in the mangrove channels, only the top meter of the water column was sampled and larvae were free to pass underneath the floating channel net. A comparison of the suites of larvae caught in each habitat would provide information about their settlement preferences. Such a comparison could not be made in the present study because the difference in the amount of the water column sampled was not controlled. However, Shenker et al. (1993) reported poor catches in subsurface deployed channel nets, and this finding indicates that most larvae that are still in the water column as they pass through the mangrove channels behind the reef crest remain near the surface of the water.

Lunar periodicity of arriving settlers has been well documented in some reef fish species; greatest recruitment usually occurs at the darkest phase of the moon (Victor, 1986; Thorrold et al., 1994; D'Alessandro et al., 2007). Rayleigh z tests on non-flow-corrected data showed that significantly more larvae were caught at the new moon in the present study. When the catch was standardized by volume of water filtered however, all lunar periods had similar numbers of individuals per unit of water volume and no periodicity existed. This finding indicates that water flow was greater during the dark moon periods (new and last quarter) than during bright moon periods (first quarter and full), and the greater water flow removed the correlation between the quantity of larvae caught and the lunar period. It appears there was approximately the same number of larval fish per unit of water volume throughout the lunar cycle; the increased flow around the new moon simply carried more of them into the nets. Alterna-

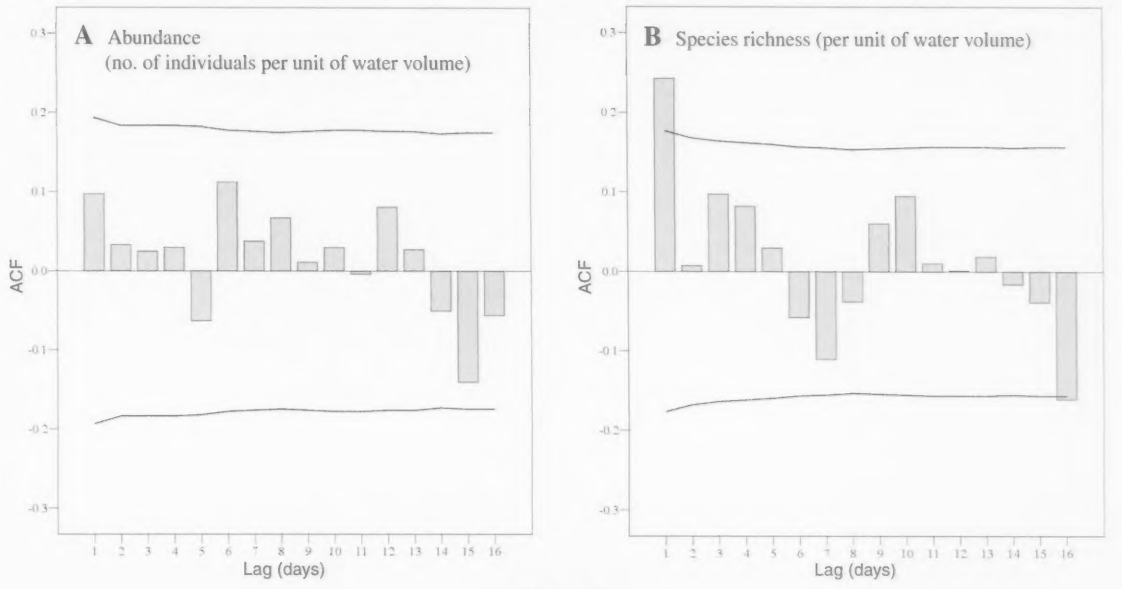

Figure 4

Auto-correlation plots of the average nightly abundance (**A**) and species richness (**B**) of larvae caught in both crest and channel nets for flow-corrected data. These plots identify any significant periodicity in the combined catch of crest versus channel nets. Auto-correlation plots are similar to cross-correlation plots but, unlike cross-correlation (which provides a comparison of two data sets), auto-correlation allows a comparison of one data set to itself. Lag refers to the number of days by which one copy of the data set is offset from the other when the correlation is calculated. Data sets are aligned for correlation on the same day at a lag of zero; one data set leads the other by one day for correlation at a lag of +1, etc. The auto-correlation function (ACF, correlation coefficient between the data sets at each lag) is on the ordinate. Values of the ACF above and below zero represent positive and negative correlations, respectively; the horizontal lines above and below the abscissa indicate the upper and lower 95% confidence limits, respectively.

tively, the larvae used this increased flow to facilitate their movement to the reef and the darker conditions to improve predator avoidance. Given that larval fish near the time of settlement possess impressive swimming and sensory abilities, the effect of flow could simply be viewed as an interesting variable that masks true larval abundance in the water column.

As reported previously (Shenker et al., 1993; Thorrold et al., 1994; Kingsford and Finn, 1997), rather than deploying a net continuously, deploying a net around the new moon with an onshore wind would optimize collection efforts. The measurements of wind speed and direction at the international airport on mainland Belize were positively correlated with abundance and species richness of fish larvae at Turneffe Atoll. Because water temperature was found to be negatively correlated with the capture of almost all species, it is possible that the emptying of warm water from the lagoon negatively affects the arrival of larvae. All of these factors (lunar period, water temperature, and prevailing wind) may be further considered when trying to optimize the collection of fish larvae in stationary nets.

In assessing the effort required to install, maintain, and deploy the codend of each type of net, we found that channel nets were far easier to work with. Because of the position of crest nets, they are subject to high wave energy and strong currents. Therefore more effort is required to anchor the frame to the substrate and more time is needed to repair the unavoidable wear and tear. Channel nets, on the other hand, are quick to retrieve in the case of a storm and require very little ongoing maintenance.

Researchers need to be aware of the additional effort required to set and maintain crest nets in comparison to other types of nets. The importance of flow has also been highlighted, and great care should be taken to evaluate this variable when making comparisons of larval catch among times and locations. Environmental factors which alter this rate of flow seem to have the greatest influence on the catch of both stationary net types. Given the results of this study, there are no obvious obstacles to the use of crest nets in other parts of the Caribbean Sea where appropriate sites exist, i.e., shallow reef crest with mainly unidirectional water flow. Given the greater water flow through the

environment in which they are deployed, they are likely to collect more larvae and hence better meet the needs of researchers working on settlement-stage reef fishes.

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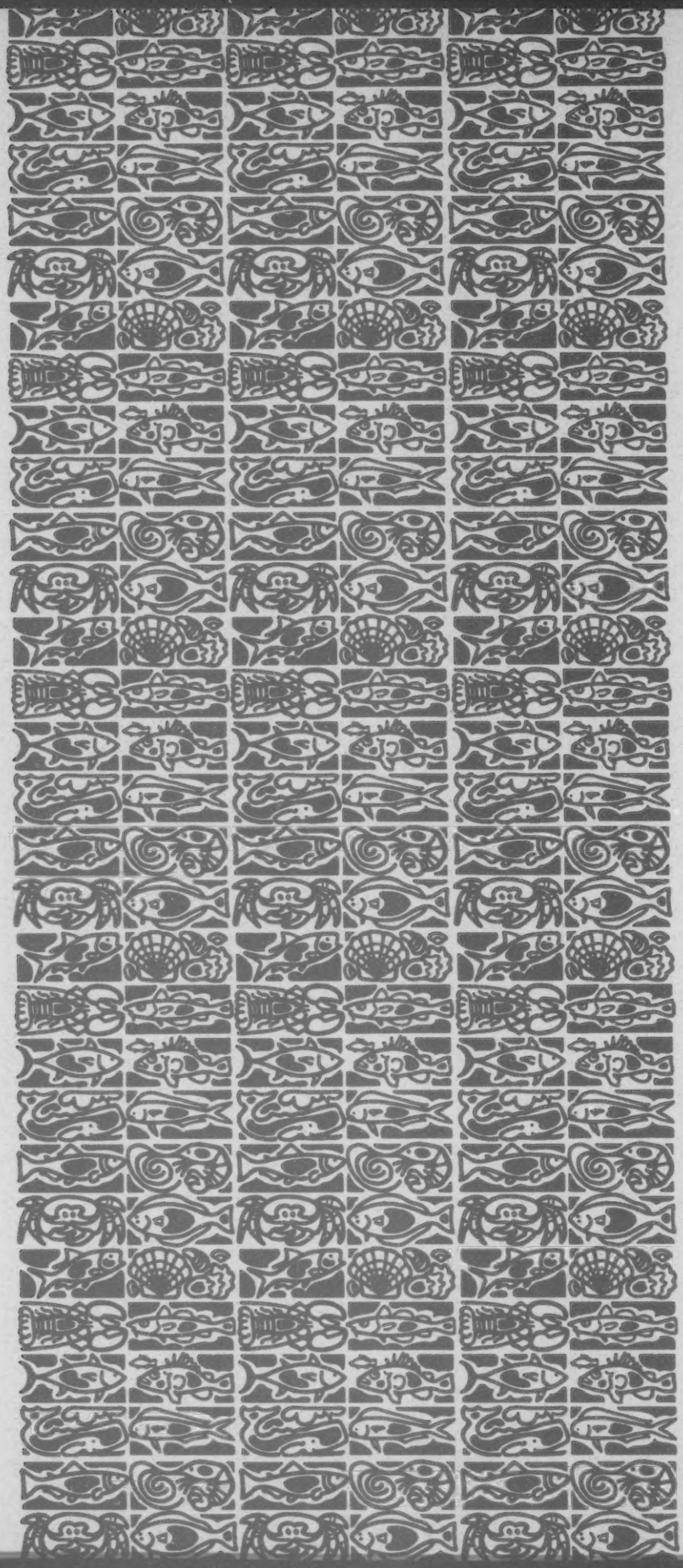
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